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SOIL FUNGI AND THEIR ACTIVITIES[1]

BY

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INTRODUCTION

It has been recognized for a long time that the soil is a favorable medium for life and life activities. While some investigators have undertaken the study of the flora of the soil proper, others took up the study of the activities of the microörganisms found in the soil, with the idea of penetrating into the secrets of the soil and thus controlling its activities. Since it is now also a well established fact that the largest quantity of plant-food applied to the soil has to undergo certain changes before it can be utilized by higher plants, and that these changes are performed by microörganisms, the knowledge of the activities of these forms of life, the ability to control them, and to direct their activities for the benefit of man, will help to solve the problem of the fertility of the soil.

The author has limited himself in this work to the fungus inhabitants of the soil. But even in this particular group of microörganisms it is impossible and not advisable to study all the forms that could be found in the soil. The question is not, what fungi can be found in the soil, but what fungi are true soil organisms, found not only occasionally and in one soil, but continually and in most soils. What part do these organisms play in the fertility of the soil? The numbers and types of fungi are usually determined, not by those that actually live in the soil, but by the germination of spores on artificial media, having poured plates of high soil dilutions. Many fungus spores germinate slowly, and a soil sample may give numerous colonies of one particular type on a plate, perhaps due to the fact that this organism happened to fruit in the soil sample, or due to the suppression of the slower germinating spores; and the particular organism may not play any great importance in the soil. Hence surveys of the soil flora have to be made repeatedly, at intervals, and this should not be limited to one or two soils, but to a wide area.

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When one speaks of microörganic activities in the soil, the word "bacteria" comes up at once, but the fact should not be forgotten that there are soils, in which the fungi also play a very important part, as is shown by their numbers and activities. The so-called "peat," "acid moor," and other similar soils, which are usually high in humus and have an acid reaction, are very rich in fungi. The mycelium of these organisms is found to penetrate the whole mass of the humus layers of the soil. The grains of a sandy humus soil are often kept tight together by the fungus mycelium. The presence of fungi is not limited merely to the surface, but penetrates deep into the soil. But, as was shown by different investigators, and as will be pointed out later, a rich fungus flora is not limited to acid soils alone, but is found also in neutral, well-aerated, and well-fertilized soils.

HISTORICAL

Adametz (1) was the first investigator who took up the study of soil fungi from a scientific point of view. He isolated his organisms from a loamy and a sandy soil, taking samples at the surface and at a depth of 25 to 30 cm., and came to the conclusion that the two soils harbored the same species, including *Penicillium glaucum, Mucor Mucedo, Mucor racemosus, Mucor stolonifer, Aspergillus glaucus* and *Oidium lactis;* in all he isolated eleven fungi and four yeasts, and did not seem to find any difference in the fungus contents of the different soil layers. Adametz, as well as those who followed him, Reinitzer (85), Nikitinsky (79), and others, studied the fungus flora of the soil for the biochemical activities of the organisms. Nikitinsky (79) isolated from the soil *Penicillium glaucum,* a Mucor and a Trichothecium. Van Iterson (52) isolated a series of fungi from the soil and from the air for the study of their cellulose decomposition, but no differentiation was made between the organisms taken from the soil and those from the air. However, among the forms that he isolated are found several Sporotricha, a Botrytis, a Mycogone, and *Cladosporium herbarum,* all of which are commonly found in the soil.

The first exact study of soil fungi, giving a classification of the organisms and full descriptions, was made by Oudemans and Koning (80) in 1902, in Holland. Forty-five species have been isolated from a humus soil, and among these organisms one finds such forms as *Trichoderma Koningi, Mucor racemosus,* and many others, which have been afterward isolated repeatedly by other investigators.

Following these preliminary investigations, of which the last one only can be considered of real scientific importance in the identification of soil fungi, comes a series of treatises on special groups of organisms. Butler (18) isolated in 1907 several Pythia from the soil, for the study of which he used an Abutillon medium, since these organisms refuse to grow readily on ordinary culture media. Hagem (42, 44) studied several

soil types, such as arable, meadow, garden, forest and other soils for their content of Mucorales. He found large numbers of these organisms in cultivated soils, though stating that in many cases they give place in numbers to the Penicillia, Aspergilli and Cladosporia. *Mucor racemosus, Mucor hiemalis* and *Mucor nodosus* were found in largest numbers. The *Mucor Ramannianus* and *Mucor silvaticus* group were found predominant in pine forests. Altogether he isolated 18 species, 8 of which were new to science. Hagem (43) stated further that the upper soil layers form a good medium for the growth of the Mucors. Some can live at as low a temperature as 6° to 8° C., and many can develop at 12° to 15° C. The upper temperature limits lie between 27° and 33° C., while the optimum is between 20° and 25° C. Moderate moisture is best for their development. The Mucors do not attack the general organic mass, the carbohydrates and cellulose; they easily attack nitrogen compounds and decompose them. Only a limited number of types assimilate nitrates and nitrites, liberating ammonia. The nitrogen compounds, such as peptones, amino acids, urea and uric acid, are very quickly utilized by the Mucors, as well as by other fungi; ammonia is formed, a part of which is assimilated and built up into fungus proteins; another and larger part does not take place in the growth of the organisms and is set free in the soil. Hagem also found that the cultivated soils vary greatly from the forest soils in their Mucorales content.

Lendner (66) made a study of the soil Mucors in Switzerland the same year as Hagem did his work in Norway. He described several species new to science and combined all the Mucors isolated before into a monograph. Following that came the work of Namyslowski (77, 78), who isolated from the soil *Zygorhynchus Vuilleminii, Mucor microsporus* and *Rhizopus arrhizus*. Ramann (83, 84) stated that he found the strongest growth of fungus mycelium in the fall, when the falling leaves afford rich food and the soil moisture is high; also that the number of fungi in the soil varies within wide limits: as a rule, in a soil that is well aerated, loose and rich in food, the bacteria play the important rôle; in the heavy soils, giving an acid reaction, the fungi predominate. Fischer (35), Hall *et altera* (45), have also pointed out the high content of fungi in the soil.

Beckwith (9), working with "wheat-sick" soils in North Dakota, found the following genera of fungi to be represented there: Fusarium, Colletotrichum, Macrosporium, Alternaria, Spicaria, Verticillium, Rhopolomyces, Cephalothecium and Helminthosporium. As will be seen later, five of these genera were found to be represented in most of the local soils studied by the writer, and had Beckwith found Aspergilli, Penicillia and Mucors, he might have had a picture of many soils, as far as the genera go. Bolley (13), in his study of "wheat-sick" soils found that certain species of Fusarium, Helminthosporium, Alternaria, Macro-

sporium, Colletotrichum and Cephalothecium are capable of destroying growing plants of wheat, oats, and other crops; this led him to believe that the sickness of the soil is caused by fungi.

Jensen (53) made a fairly complete study of the fungus flora of the soil, taking his samples under sterile conditions and using several soil types and through several seasons of the year. He isolated 35 species, many of which were also reported by previous investigators. Among these are found some of the most common soil groups, such as the Mucors, Penicillia, Aspergilli, Cladosporia, etc. Jensen (53) laid special emphasis upon the occurrence of facultative parasites in the soil.

Dale (27) studied the fungus flora of sandy, chalky, peat and "black earth" soils. The curious thing is that she found in the soil many organisms, such as *Trichoderma Koningi,* several Mucors, Penicillia, Cladosporia and others, which Hagem (42) isolated from the soil in Norway, Koning (80) in Holland, and Jensen (53) in this country. Many of the genera and even the species are the same. She isolated altogether over one hundred organisms, many of which were found in all or in several of the soils.

Goddard (41) isolated 18 species of fungi from the soil for the study of their nitrogen-fixing power. Seven of these organisms are the same as those found by Koning (80). He tried to make a study of the distribution of fungi with soil depth. His results indicate that the organisms were distributed rather uniformly at different depths, at least as low as 14 cm. As to the occurrence of the particular species at the different depths, he did not discover any definite relationship,—any particular species seemed about as abundant at one depth as at another. He was inclined to conclude that there is a rather constant and characteristic fungus flora in the soil, regardless of the treatment as to tillage or manuring.

McLean and Wilson (73) have isolated from a New Jersey Sassafras loam, with an acid reaction, 26 species of fungi, among which are found many organisms reported by the investigators previously mentioned. Finally, mention has to be made of the work of Traaen (108) in Norway, who isolated over 100 fungi from the soil, using filter paper as a substratum. One gets a very faint idea about the organisms that he found, since no identification has been undertaken. He related most of the organisms found to two new genera, subdivided into several species. Of the well known organisms he mentions *Trichoderma lignorum, Trichoderma Koningi* and *Mucor spinosus.*

As seen from the historical review on the occurrence of fungi in soils, the numbers and types of these organisms are large enough to warrant the idea that they play an important part in the fertility of the soil, but to what extent? Are their activities limited to one particular field, or do they take part in the several soil processes which are necessary for the making of a fertile soil? A great deal of work has been done on the

activities of fungi, as factors in the fertility of the soil, and though some of it was done with organisms isolated from the air or other sources, since these are also found in the soil the work accredited to them may also be understood as taking place in the soil.

The field of fungus activities in the soil can be discussed under the following four headings: first, ammonification; second, nitrogen-fixation; third, nitrogen-transformation; and fourth, cellulose decomposition and humification.

Müntz and Coudon (76) were the first ones to demonstrate the ammonifying power of the several soil organisms, including bacteria and fungi, and it is interesting to note that the two fungi used, namely, *Mucor racemosus* and *Fusarium Müntzii*, gave larger ammonia accumulation in soil than any bacterium used; the same held true in bouillon, with one exception, where one bacterium gave a larger amount of ammonia than the Fusarium.

Marchal (71) proved soon afterward that fungi, such as *Aspergillus terricola, Cephalothecium roseum* and others, are active ammonifiers, especially in acid soils. Butkewitsch (17) pointed out the fact that *Aspergillus niger* is a strong ammonifying organism. Decomposition of urea by fungi has been observed by Miquel (74), Semal (97), and Shibata (99). Perotti (81) found that pure cultures of *Aspergillus fumigatus, Aspergillus ochraceus, Aspergillus niger, Penicillium glaucum, Mucor Mucedo,* and *Botrytis cinerea* showed a good development in dicyanamide solutions, where this was the only source of nitrogen. Kappen (55) found that *Penicillium brevicaule, Stysanus stemonitis,* and two other fungi, were able to decompose cyanamide into ammonia. But dicyanamide could not be utilized hy those organisms as a source of nitrogen.

Hagem (43) stated that when ammonia salts are found in the soil, they are quickly utilized and converted into fungus protein,—thereby larger or smaller quantities of easily assimilated nitrogen compounds are taken away from the higher plants and converted into a form which they cannot utilize. In this case the activity of the soil fungi is throughout injurious for the green vegetation. But in the case of dead plants and animal remnants, complicated organic compounds, the soil fungi play a very useful part: they decompose them, liberating ammonia, a part of which may be utilized by them; the larger part, however, is set free, and after being nitrified it can be utilized by the green plants. He ascribed to the soil fungi an important part in the mineralization of organic matter, in which they are greatly ahead of the bacteria.

McLean and Wilson (73) tried out the ammonifying power of a series of fungi isolated from the soil, and concluded that fungi rather than bacteria were responsible for the large accumulation of ammonia under the conditions with which they were working. The Moniliaceae were found to be the strongest ammonifiers, the Aspergillaceae showing the least ammonifying activity. Waksman and Cook (115) thought that

these differences in the ammonifying power of fungi might be due not so much to the organisms themselves, as to the incubation period used. The Moniliaceae, especially *Monilia sitophila,* producing spores very readily, decompose the organic matter and liberate the ammonia at an earlier period than do the Aspergillaceae, which produce their spores after a longer period. They expressed the idea that there might be some relationship between the periods (e. g. spore production) in the life history of the fungus and the ability to accumulate ammonia in the soil.

The effect of reaction upon ammonification by soil fungi has been recently studied by Kopeloff (60), who found that there exists a comparatively narrow range of reaction tolerance for maximum ammonification, which was found to be between the neutral point and an acidity equivalent to 2000 pounds of CaO per acre.

As to the fixation of atmospheric nitrogen by fungi, a great deal of work has been done on the subject, but the results are still indefinite. Frank (38), Berthelot (12), Puriewitch (82) Saida (91), Remy (87), Ternetz (103), Froelich (40), Latham (64), C. B. Lipman (69), Stahel (101), and Heinze (47), have all shown that fungi are able to utilize free nitrogen. Winogradsky (125), followed by Czapek (26), reported negative results with Aspergilli, and the latter pointed out the possibility of an error in the nitrogen determinations made by Saida (91). Negative results were also reported later by Heinze (49). Duggar and Knudsen (30) found no nitrogen-fixation by fungi, except possibly in certain cultures of *Aspergillus niger.* Kossowicz (61), having tried out the nitrogen-fixing power of a series of fungi, including *Aspergillus niger* and *Penicillium glaucum,* found that they were able to utilize the nitrogen compounds of the air, but were not able to assimilate any free atmospheric nitrogen. Goddard (41) has done about as complete and exhaustive a piece of work on the subject as any previous investigator. He reports negative results for all the forms studied, including at least 14 species, when grown on nitrogen-free media; the amount of free nitrogen taken up from the air by cultures standing exposed, did not seem to be sufficient to make appreciable differences in their nitrogen content, either in nitrogen-free or in nitrogen-containing media. The weight of the conclusions on the nitrogen fixation by fungi seems to lie on the negative side.

The possibility of nitrogen-fixation by another group of soil organisms closely related to the soil fungi, namely, the soil algae, alone, or in association with fungi, has been studied by Schlösing, Jr. and Laurent (96), Kossowitsch (62), Koch and Kossowitsch (57), Stoklasa (102), Beijerinck (10), Heinze (48), and Wilfarth and Wimmer (124).

There is a group of soil fungi for which the ability to utilize atmospheric nitrogen seems to be more or less affirmative, namely, the mycorhizal fungi, but since these organisms do not come within the field of the soil fungi proper, the author will merely refer to the work of Frank

(36, 37) and Hiltner (50), where references are also given to the other workers on the endotrophic and ectotrophic mycorhiza.

Not a very great deal of work has been done on the utilization of the different forms of nitrogen by fungi, and the transformation of nitrogen compounds. Ritter (89) found that *Aspergillus glaucus, Mucor racemosus,* and *Cladosporium herbarum* can use ammonia nitrogen as well as nitrate nitrogen. Ehrenberg (31) called attention to the fact that soil fungi play a more important part in the building of albuminoids from ammonia nitrogen than the bacteria do. Laurent (65) found *Cladosporium herbarum, Penicillium glaucum, Alternaria tennis,* and *Mucor racemosus* able to reduce nitrates to nitrites; this did not hold true with *Aspergillus glaucus* and *Aspergillus niger.* But no direct denitrification could be established with fungi, as it is done with bacteria. Ehrlich and Jacobsen (32) found that *Oidium lactis, Monilia sitophila,* Mucors, and other fungi can form oxy-acids from amino-acids.

A great deal of work has been done on the decomposition of carbohydrates by fungi, including celluloses, hemicellulose, pectins, oils, fats, and acids; also on the building up of those complicated compounds in the soil termed humus. Fremy (39) showed in 1848 the ability of *Mucor stolonifer* to destroy pectin. De Bary (8) found that *Peziza libertiana* is able to decompose cellulose. This was also found to hold true for different other fungi by Ward (116, 117), Appel (2), and other investigators which have studied the pathogenicity of certain fungi. Van Iterson (52) distinguishes four ways in which cellulose can be decomposed: by anaerobic bacteria, denitrifying bacteria, aerobic bacteria, and fungi. Among the strongest cellulose decomposers of the 35 distinct species that were able to live on cellulose, he found *Mycogone puccinoides, Botrytis vulgaris,* and a few others. Went (122) found that *Monilia sitophila* is able to secrete cytase. Carbone and Marincola (20) observed the cellulose decomposition by Eumycetes. Carbone (19) found that the Penicillia decompose cellulose; the same thing was pointed out by Christensen (22).

Schellenberg (94) could not find any decomposition by fungi of true cellulose, but found them able to decompose hemicellulose. Dox and Neidig (29) have shown that *Aspergillus niger, Aspergillus fumigatus, Aspergillus clavatus, Penicillium chrysogenum, Penicillium camemberti,* and *Penicillium expansum* are able to destroy the pentosans found in large quantities in the soil and manure. Ohta Kohshi (58) found *Cladosporium herbarum, Penicillium glaucum, Aspergillus glaucus, Aspergillus nidulans* to be able to decompose fats. Roussy (90) proved that fatty acids, namely oleic and palmitic, can be used by fungi as good sources of food.

As to the humifying power of fungi, Frank (37) was the first one to point out the fact that the forest humus is not to be looked upon as a mass of plant remnants, but it is largely a living mass of numerous fun-

gus hyphae which penetrate in all directions and often make up an important part of the organic substance. They take an active part in the decomposition of organic substances. Höveler (51) looked upon the fungi as the principal factors in humification. Kostystscheff (63) stated that the microörganisms, including bacteria and fungi, help in the formation of the brown substance of the humus. A similar statement concerning the fungi was made by Scherpe (95). Beijerinck (11) expressed the opinion that the formation of humus in the fields and forests is based upon the equilibrium of oxidations and reductions produced by microorganisms. If oxidation predominates, the humus may disappear; but, if reduction predominates, the soil loses its fertility by the formation of peat.

Koning (59) stated that the Hyphomycetes play a much more important part in the process of humus formation than do the bacteria. He inoculated *Trichoderma Koningi* and *Cephalosporium Koningi* upon sterile leaves covered with a little water. At the end of several weeks the leaves disappeared and the water contained NH_3. Upon the addition of KOH and NH_3 it became dark, as do the humic substances. He further stated that these organisms produce cytase, called also cellulase, which allows them to utilize the cellulose as food. The liquid of the culture gave a faint reaction with Fehling's solution, which led him to conclude that sugar is formed as an intermediate product.

Dascewska (28), having studied both bacteria and fungi from the soil, came to the conclusion that the Hyphomycetes play a more important rôle in the decomposition of cellulose in the soil than do the bacteria. The dark color of humus is probably due, at least in part, to the color of the mycelium and the spores, to brown and black pigments, as well as to oxidizing substances secreted mostly by the Hyphomycetes.

McBeth and Scales (72) concluded that the importance of the filamentous fungi in the soil in the destruction of cellulose has been greatly underestimated. They found that a series of Penicillia, Sporotricha, and other fungi commonly found in the soil were able to decompose different forms of cellulose very rapidly. Scales (93) found that a great number of Penicillia and Aspergilli were able to decompose cellulose, especially where ammonium sulphate has been used as a source of nitrogen.

Heinze (47) stated that soil fungi, the so-called acid builders, play an important part in the making of the insoluble Ca and Mg compounds, soluble and available for plant growth; a similar effect is produced upon the insoluble phosphates, such as Thomas slag, which are made slowly soluble and easily available. A like idea is expressed by Hagem (43), as was already pointed out.

Thus the activities of fungi are found to be manifold; they take an important part in most of the soil processes which work for the keeping up of the fertility of the soil. There are few processes in the soil, such

as nitrification and nitrogen-fixation, in which the fungi were not found to take an important part, or in which their rôle is doubtful. The problem of the author was to isolate all the fungi from a series of soils and then study their activities, since a great deal of the work reviewed in the previous pages was done with organisms either isolated from the air or living parasitically on plant tissues.

EXPERIMENTAL.

Soils Used

Eight soils were used for this work, which will be designated in the following manner:

1. *Garden soil,* a Sassafras sandy loam, on the College Farm, manured every year for the last 20 years with 20 tons of stable manure annually, and receiving an application of lime every 5 years. This soil is almost neutral, showing a lime-requirement, by the Veitch method, of 200 pounds of CaO per acre at 1 inch from the surface, 100 pounds at 4 inches. Below that depth the soil is almost neutral or even slightly alkaline. This soil is used for garden crops by the Botanical Department for experimental purposes.

2. *Orchard soil,* a Sassafras sandy loam, near the garden soil, of exactly the same texture and structure. This soil is the unfertilized plot of the orchard, receiving no application of manure or fertilizer for the last 20 years; only a cover crop of oats has been grown for the last 3 years. Apple trees are grown on this soil, and it receives 8 to 10 cultivations through the summer. This soil has a lower organic matter content than the garden soil, and a high lime-requirement, 2500 pounds CaO per acre at 1 inch from the surface.

3. *Meadow soil,* an Alloway clay, under grass for the last 6 years. This is a heavy soil, with a high moisture, nitrogen, and organic matter content. The lime-requirement is about 500 pounds CaO per acre.

4. *Forest soil,* of the same type as the first two soils, but not cultivated for the last 50 years, if ever before. It contains a large amount of undecomposed organic matter, of an acid character, in the upper 4 inches of soil. Below that depth the soil is very poor in food nutrients, though remaining high in acidity. The lime-requirement of this soil is 4600 pounds of CaO per acre at 1 inch from the surface.

These four soils were the standard soils, from which the samples were taken regularly, under exact methods and at known depths. The description of soil sampling, bacteriological, climatological, and other data will apply to these soils only.

Four more soils have been used for this work, but the data on those soils were merely supplementary, and no exact study has been made of the soils themselves.

5. *Sassafras gravelly loam,* a medium loamy soil, under irregular rotation of crops.

6. *Iron soil,* a medium loam termed "Colts Neck loam," containing 48 per cent of iron, which gives to the soil a brick-red appearance. This soil is from a peach orchard, near Keyport, N. J.

7. *California soils,* of different texture and structure, taken near Los Angeles.

8. *Oregon soil,* an adobe soil at Corvallis, Oregon.

Samples were taken in all cases under sterile conditions, brought at once into the laboratory, allowing the time of shipment for the last three soils, and plated out on the media used. Care was taken to avoid all possible contaminations from the air, using sterile dishes and sterile water for dilutions. Samples were taken 4 times in the case of the first four soils, namely, September 10, October 21, November 30, 1915, and January 4, 1916, at depths of 1, 4, 8, 12, 20, and 30 inches. The full method of procedure is described elsewhere (113). Samples were taken only once in the case of the last four soils, at a depth of 8 to 10 inches.

Media Used

In choosing the media for the work, the author did not want to make up any new ones, but to select from those used by other investigators such as would give the best results, and that the work might be comparable with that of others. The fault of devising new media every time an experiment is made is great, when one understands the fact that most of the indentification to be done is from descriptions, not comparison of cultures, and since some organisms vary so much with the different media used, one would have to use all the media ever thought of by the different investigators to have results fully comparable. A synthetic medium is wanted, which would have a known chemical composition, and which would be the same wherever used. Such a medium was found in Czapek's agar, used by several investigators, and recommended to the author by Dr. Charles Thom, of the Bureau of Chemistry, U. S. Department of Agriculture. Though many organisms grow very slowly on this medium, the final growth will be characteristic, and a medium which allows a slower development of the organisms is more desirable for the study of the gradual development of the organisms. Other media may be used for supplementary purposes, but one should be used as a standard, with which all the results could be compared.

Four media were used in this work:

1. *Modified Albumen Agar* (16, 113), for the plating out of the original soil samples. It is composed as follows:

Distilled water	1000 c.c.
Dextrose	10.00 gm.
K_2HPO_4	0.50 gm.
$MgSO_4$	0.20 gm.
Egg-albumen	0.15 gm.
$Fe_2(SO_4)_3$	Trace
Agar	15.00 gm.

This medium was used, not because it is the best one for the isolation of fungi, but for several other reasons: (a) It allowed the greatest development of both fungi and bacteria, under the given conditions; this gave a means of comparison of the numbers of the two most important groups of organisms in the soils used, under absolutely the same conditions. (b) It is a medium rather poor in plant-food, thus allowing only a very slow growth of all the organisms. This made it possible to transfer the organisms when 8 to 10 days old, so that even the slow growing fungi could be isolated without being covered up by the rapidly growing ones. The egg-albumen was dissolved in a little cold water to which a few drops of NaOH were added, and this was added to the hot medium already prepared and well shaken. No coagulation of the albumen will take place upon sterilization. (c) It allows a better development of all different groups of organisms than do the other media, with which this one was compared. (d) The conditions were more uniform when both bacteria and fungi, slowly and rapidly growing forms, could be studied on the one plate. However, no identification was made from this medium, and it was used only for isolation purposes.

2. *Raisin Agar.* This medium was used for the purification of fungi contaminated with bacteria. It was prepared as follows: 60 gm. of yellow raisins were warmed at about 80° C. in 1 liter of tap water for one hour, then filtered, and all the liquid pressed out from the raisins. The filtered portion was made up to 1 liter and 25 gm. of agar were dissolved in it. The medium was then tubed and sterilized. This medium is acid in character, and keeps out the bacteria, thus giving cultures of fungi free from bacteria.

3. *Cook's No. II Medium* (24), composed as follows:

Distilled water	1000 c.c.
Dextrose	20.00 gm.
Peptone	10.00 gm.
K_2HPO_4	0.25 gm.
$MgSO_4$	0.25 gm.
Agar	15.00 gm.

This medium was used for the study of the Mucorales, most of which do not make any good growth upon Czapek's agar.

4. *Czapek's Synthetic Solution Agar,* composed as follows:

Distilled water	1000 c.c.
$MgSO_4$	0.50 gm.
K_2HPO_4	1.00 gm.
KCl	0.50 gm.
$FeSO_4$	0.01 gm.
$NaNO_3$	2.00 gm.
Cane sugar	30.00 gm.
Agar	15.00 gm.

All the organisms, with the exception of the Mucorales, have been studied upon this medium.

In the process of isolation of the fungi from the soil two methods of procedure have been followed:

1. *The dilution method.* The soil samples, having been taken in sterile flasks, under sterile conditions, at the desired depths, were brought at once into the laboratory. The soil was well mixed and a piece of it was transferred with a sterile spatula into a weighed sterile flask. The flask was then weighed again, and the weight of the transferred soil was found by difference. Dilutions were made up to 2,000, 20,000, and 200,000, sterile water being used. Plates with the albumen agar as the medium were poured from the different dilutions. The plates were then incubated at 22° to 25° C. for 6 to 10 days, after which period the bacteria and fungi were counted, and the fungi were transferred to slants of Czapek's agar with a sterile needle.

2. *The direct inoculation method* (114). Lumps of soil, about 1 c.c. in diameter, were transferred from the sampling flask, with a sterile forceps, into a sterile plate containing 10 c.c. of Czapek's agar. The plates were then allowed to incubate for 24 hours at 22° C. It was believed that in such a short period of time the spores in the soil could not have germinated and formed a mycelium, visible to the naked eye; while the organisms actually living in the soil and forming a mycelium would develop at once and the mycelium would then be visible to the naked eye. After 24 hours of incubation the mycelium was transferred from the plates into sterile slants of Czapek's agar, care being taken to get the mycelium from the medium 1 to 3 mm. away from the soil lump.

The tube cultures from both isolations were allowed to develop for 10 to 15 days, till spore production took place. Single spore cultures were made then, using the raisin agar as the medium for transfer, thus eliminating the bacteria. A small amount of spores and mycelium from the organism in question were introduced into 100 c.c. of sterile water in a 300-c.c. flask. This was well shaken so as to break up the spore material. A platinum needle was then sterilized, dipped into the liquid, and passed several times over the sterile solidified medium in the sterile plate. The plates were then incubated 2 to 3 days till the colonies began to develop. Where the single spores were dropped by the needle, separate colonies could be detected with the naked eye. This was verified by placing the plate, with the lower side upward, under the microscope. The single spore colony, or part of it, was now transferred with a platinum loop into a sterile slant of No. II medium in the case of Mucorales, or Czapek's agar in the case of any other fungus. The tubes were incubated at 22° to 25° C. and growth compared with original growth on Czapek's agar, so as to get the identity of the culture. The last transfer usually gave a culture free from any bacterial or fungus contamination.

Certain organisms, especially a few Penicillia, always came out from this transfer contaminated with a sterile mycelium, and it took several transfers before such could be had pure.

All the identification and description was done on the last transfer. In the identification of the Penicillia, 15 per cent gelatin in distilled water was also used, as recommended by Thom (104). The media used for cellulose decomposition and diastase secretion by fungi will be described later.

Numbers of Fungi in the Soil

Remy (86), who took his soil samples in different seasons of the year, recorded both the bacteria and fungi developing on the plates. He found no constant ratio between the numbers of the two groups of microörganisms. But on the average he found in cultivated soils ten times as many bacteria as fungi; 1,770,000 to 2,500,000 bacteria and 220,000 to 240,000 fungi were found per gram of soil.

Ramann (83) found an average of 2,400,000 bacteria and 129,000 to 289,000 fungi per cubic centimeter of loamy and sandy soils; in raw humus soil he found 220,000 bacteria and 241,000 fungi per cubic centimeter. Fischer (35) found almost as many fungi as bacteria in cultivated and uncultivated moor lands, but in clay, sandy, and loamy soils the numbers of fungi were much smaller than those of bacteria. In untreated moor land he found an average of 1,600,000 bacteria and 550,000 fungi per gram; when this soil received an application of stable manure, it contained 700,000 bacteria and 1,116,666 fungi per gram. Sandy soil untreated contained 1,450,000 bacteria and 733,333 fungi per gram; manured, 3,650,000 bacteria and 1,816,666 fungi. The writer (112) reported in a previous paper, 6.2 to 7.1 per cent of the total microörganic flora to be fungi, which amounted to 400,000 to 1,100,000 per gram of soil at a depth of 1 inch, 7.9 to 11.7 per cent at a depth of 4 inches, and at lower depths the total number of fungi, as well as their relation to the bacteria and actinomyces, decreased with depth.

As seen from Table I, the cultivated and non-acid soils contain large numbers of fungi, and even larger than those of the forest soil, which is very acid. The numbers of bacteria for those soils throughout the whole year have been reported elsewhere (113). The first three soils were found to contain from 4,000,000 to 20,000,000 bacteria, while the forest soil contained only 500,000 to 4,000,000. The ratio of the bacterial to the fungus numbers in the first three soils was about 10 to 1, while in the forest soil it was about 5-6 to 1, and sometimes the forest soil contained almost as many fungi as bacteria, especially when the medium was slightly acid. When the numbers of fungi found at the different depths are compared, the highest numbers are found at 1 or at 4 inches from the surface. The garden soil contained in three instances out of four larger

numbers of fungi at a depth of 4 inches than at 1 inch. This was probably due to the high organic matter and moisture content of the lower depth. Below 4 inches the numbers decrease rapidly with depth, the greatest fall usually occurring between 4 and 8 inches. At depths of 20 and 30 inches the numbers of fungi were very small, so that on a 10,000 to 20,000 dilution plate only one or two colonies would develop, and sometimes none at all. This does not agree with Goddard's (41) remark, that the species seemed to be as abundant at one depth as at another.

TABLE I

NUMBERS OF FUNGI PER GRAM OF SOIL AT DIFFERENT DEPTHS[1]

Date of sampling	Depth in inches	Garden soil			Orchard soil			Meadow soil			Forest soil		
		Numbers	Numbers	Average	Numbers	Numbers	Average	Numbers	Numbers	Average	Numbers	Numbers	Average
Sept. 10, '15	1	400	600	500	400	600	500	800	1,000	900	240	200	220
"	4	800	1,000	900	600	1,000	800	1,400	1,200	1,300	160	130	145
"	8	300	200	250	180	120	150	300	200	250	...	...	...
"	12	160	130	145	50	40	45	60	40	50	...	...	...
"	20	60	40	50	30	20	25	30	30	30	...	...	...
"	30	30	20	25	20	10	15	30	20	25	...	...	...
Oct. 21, '15	1	400	400	400	500	400	450	1,200	1,000	1,100	180	240	210
"	4	800	600	700	400	600	500	600	800	700	180	200	190
"	8	600	400	500	100	80	90	120	140	130	...	...	...
"	12	60	40	50	40	20	30	40	40	40	...	...	...
"	20	40	20	30	20	20	20	40	60	50	...	...	...
"	30	40	40	40	0	0	0	20	20	20	...	...	...
Nov. 30, '15	1	200	100	150	400	400	400	600	600	600	240	180	210
"	4	400	200	300	200	100	150	600	800	700	80	100	90
"	8	100	0	50	120	60	90	200	100	150	...	...	...
"	12	40	50	45	60	40	50	60	50	55	...	...	...
"	20	20	30	25	20	20	20	20	10	15	...	...	...
"	30	10	0	5	20	0	10	0	0	0	...	...	...
Jan. 4, '16	1	200	200	200	200	100	150	800	1,000	900	260	200	230
"	4	300	100	200	100	0	50	400	200	300	220	140	180
"	8	120	60	90	40	20	30	200	200	200	...	...	...
"	12	40	40	40	10	0	5	40	40	40	...	...	...
"	20	20	20	20	0	0	0	6	6	6	...	...	...
"	30	2	2	2	0	0	0	2	0	1	...	...	...

[1] Thousands omitted.

The duplicates of the fungus counts checked very poorly, one plate giving 6 to 10 colonies, and the duplicate only 3 to 4 colonies. This might have been due to the fact that the spore material was not broken up well enough when the dilution was made. As to the seasons of the year, no appreciable difference in the fungus content of the soil was found in the different periods of sampling.

In Table II are given all the fungi isolated from the different soils, with special emphasis laid upon the four standard soils. Several more organisms are kept in culture, but the author has not been able to identify

TABLE II

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENT SOILS

Name of Organism	Garden	Orchard	Meadow	Forest	Iron	Oregon	California	Sassafras
Absidia Lichtheimi (Lucet et Const.) Lendner	*	..	..	..	..	..	..	..
Absidia Orchidis (Vuill.) Hagem	..	..	..	..	*	..	..	..
Mucor hiemalis Wehmer	*	*	..	*	..	..	..	*
Mucor microsporus Namyslowski	..	*	..	..	..	..	..	..
Mucor circinelloides van Tieghem	*	*	*	*	..	..	..	..
Mucor plumbeus Bonorden	*	*	*	*	..	*	..	..
Mucor racemosus Fres.	*	*	*	..	..	*	..	..
Mucor silvaticus (?) Hagem	..	*	..	..	..	..	..	..
Mucor botryoides Lendner	..	..	..	..	..	..	..	*
Mucor saturninus (?) Hagem	..	*	..	..	..	..	..	..
Mucor sphaerosporus Hagem	..	*	..	..	..	..	..	..
Mucor sp. (C. 44)	..	..	*	..	..	..	..	..
Mucor sp. (D. 28)	..	..	..	*	..	..	..	..
Mucor glomerula (Dainier) Lendner	..	..	*	..	*	..	..	..
Mucor flavus Bainier	..	..	..	..	*	..	..	..
Zygorhynchus Vuilleminii Namys.	*	*	*	..	*	*	*	..
Rhizopus nigricans Ehren.	*	*	*	..	..	*	..	..
Rhizopus nodosus Namys.	..	*	*	..	..	..	..	..
Rhizopus sp. (B. 3)	..	*	..	..	..	..	..	..
Saccharomyces sp.	*	*	*	*	..	..	..	..
Chaetomium olivaceum Cooke and Ellis	..	..	*	..	..	..	..	..
Chaetonium cochliodes Palliser	..	..	..	..	..	..	..	*
Monilia sitophila (Mont.) Sacc.	*	..	*	*	..	..	..	..
Monilia humicola (?) Oud.	..	..	*	..	..	..	..	..
Oidium lactis Fres.	*	*	*	..	..	..	..	..
Oidium sp.	*	..	..	..	..	..	..	..
Sporotrichum pulvinoforme (?) Thüm	..	..	..	..	*	..	..	..
Sporotrichum roseum (?) Link	*	..	..	..	..	..	..	..
Botrytis cinerea Pers.	..	..	*	..	..	..	..	..
Aspergillus fumigatus Fres.	*	*	*	*	..	*	..	..
Aspergillus nidulans Eidam	*	*	*	*	..	..	..	..
Aspergillus diversicolor Vuill.	*	*	*	*	*	..	*	..
Aspergillus niger van Tieghem	*	*	*	..	..	..	..	..
Aspergillus n. sp. (?) (C. 19)	..	..	*	..	..	..	..	..
Aspergillus glaucus Link	..	..	*	..	..	..	..	..
Aspergillus calyptratus (?) Oud.	..	..	..	..	*	..	..	..
Aspergillus fuscus Schiemann	*	..	..	..	..	..	..	..
Aspergillus flavus Link	..	..	..	..	..	..	*	..
Aspergillus sp. (K. 2)	..	..	..	..	..	..	*	..
Aspergillus repens de Bary	..	..	..	*	..	..	..	..
Aspergillus clavatus Desmasières	..	..	..	..	..	..	..	*
Penicillium luteum Zukal	*	..	..	..	..	..	..	..
Penicillium luteum group Thom	*	..	*	..	*	..	..	..
Penicillium lilacinum Thom	..	..	*	..	..	..	..	..
Penicillium pinophilum (Hedgcock) Thom	..	..	*	..	..	..	..	..
Penicillium purpurogenum, var. *rubrisclerotium* (?) Thom	..	..	*	..	..	..	..	..
Penicillium chrysogenum Thom	..	..	*	..	..	..	*	*
Penicillium commune Thom	..	*	..	..	..	..	..	..
Penicillium decumbens Thom	..	..	..	*	..	..	..	..
Penicillium digitatum Sacc.	*	..	..	*	*	..	..	..
Penicillium expansum (Link) Thom	..	..	..	*	..	..	..	..
Penicillium italicum Wehmer	..	..	..	..	..	..	*	..
Penicillium oxalicum Thom	*	..	..	..	..	..	..	..
Penicillium notatum Westling	*	..	*	*	..	..	..	..

TABLE II—(Continued)

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENE SOILS

Name of Organism	Garden	Orchard	Meadow	Forest	Iron	Oregon	California	Sassafras
Penicillium viridicatum Westling	*	*	..	..	*	..	..	..
Penicillium atramentasum Thom	*	..	..	..	..	..	..	..
Penicillium glaber[1] Wehmer	*	*	..	*	..	*	..	..
Penicillium Pfefferianus[1] Wehmer	..	..	..	*	..	*	..	..
Penicillium rugulosum Thom	..	..	..	*	..	..	..	..
Penicillium lividum Westling	..	..	..	*	..	..	..	..
Penicillium cyclopium Westling	..	..	..	*	..	..	..	..
Penicillium desiccens Oud.	..	..	*	..	..	..	..	..
Penicillium group I (13-25)	*	*	*	*	*	*	*	..
Penicillium group II (6-22-23)	..	..	*	*	..	*	..	..
Penicillium group III (9-24)	..	..	*	*	..	..	..	..
Penicillium group IV (2-11-15)	..	*	*	..	..	..	..	*
Penicillium group V (18-20)	..	*	..	*	..	..	..	..
Penicillium group VI (10)	*	..	..	..	*	..	..	..
Scopulariopsis brevicaule Sacc. (n. var.?)	*	..	..	..	*	..	..	..
Cephalosporium acremonium Corda	..	..	*	..	..	..	..	..
Cephalosporium curtipes (?) Sacc.	..	..	*	..	..	..	..	..
Cephalosporium sp. (G. 23)	..	..	..	..	*	..	..	..
Cephalosporium sp. (D. 32)	..	..	..	*	..	..	..	..
Cephalosporium sp. (C. 56)	..	..	*	..	..	..	..	..
Trichoderma Koningi Oud.	..	..	*	*	*	*	..	..
Trichoderma lignorum (Tode) Hartz	..	..	*	*	..	..	..	..
Trichoderma sp. (G. 5)	*	..	..	*	*	..	..	..
Trichoderma sp. (C. 10)	..	..	*	*	..	..	..	..
Trichoderma sp. (D. 34)	..	..	..	*	..	..	..	..
Acrostalagmus cinnabarinus, var. *nana* Oud.	*	*	*	..	..	*	..	..
Acrostalagmus albus Preuss.	*	..	*	..	..	..	..	..
Verticillium glaucum (?) Bonorden	..	..	..	*	..	..	..	..
Verticillium terrestre (?) (Link) Lindau	..	..	..	*	*	..	..	..
Cephalothecium roseum Corda	..	..	*	..	..	..	..	..
Zygodesmus sp. (B. 36)	..	*	..	..	..	*	..	..
Dicoccum asperum Corda	*	..	..	..	..	..	..	..
Basisporium gallarum (?) Molliard	..	..	..	..	*	..	..	..
Cladosporium herbarum Pers.	*	*	*	*	..	*	..	..
Cladosporium epiphyllum Pers.	*	..	*	..	..	..	*	..
Dematium pallulons (?) de Bary	..	.	..	..	..	..	*	..
Acremoniella sp. (?) (C. 37)	..	..	*	..	..	..	..	..
Alternaria humicola (?) Oud.	..	..	*	..	..	..	..	..
Alternaria sp. (B. 20)	..	*	..	..	..	..	..	..
Alternaria sp. (A. 36)	*	..	*	..	..	..	..	..
Fusarium angustum Sherb.	..	..	..	..	..	..	..	*
Fusarium bullatum[2] Sherb.	..	..	..	..	..	..	..	..
Fusarium solani[2] Ap. et Wr.	..	..	..	..	..	..	..	..
Fusarium orthoceras Ap et Wr.	..	*	..	..	..	..	..	..
Fusarium oxysporium var. *resupinatum* Sherb.	..	..	..	*	*	..	..	..
Fusarium caudatum Wr.	..	..	..	*	..	..	..	..
Fusarium oxysporium Schlech.	..	..	..	..	*	..	..	..
Melanconium sp. (G. 20)	*	..	..	..	*	*	..	..
Coniothyrium Fuckelii (?) Sacc.	..	..	*	..	..	..	..	..
Sclerotium	..	..	..	*	..	*	..	..
Sterile white mycelium	*	*	*	*	*	..	..	..
Sterile red-brown mycelium	*	..	..	*	..	*	..	..

[1] Organisms belong to Wehmer's genus Citromyces.

[2] Isolated from an alfalfa soil not given in the table.

them or to determine with any accuracy the species or genus which they approach. Such, for example, are several Mucors, Rhizopuses, Fusaria, Alternaria, Sclerotia, white sterile mycelium producing only chlamydospores, and others.

TAXONOMIC CONSIDERATION OF ALL FORMS FOUND IN THE SOIL

In the identification of the fungi much assistance has been found in Rabenhorst's "Kryptogamen Flora" (34, 68), Engler and Prantl's (33) work on fungi, Thom's (104) and Westling's (123) work on Penicillia, Hagem's (42, 44) and Lendner's (66) work on the Mucorales, and a number of papers on fungi in general and soil fungi in particular, such as the work of Dale (27), Jensen (53), Oudemans and Koning (80), and many others. The measurements are always recorded as found by the author; these in many cases did not coincide with those given in the original description. The identifications being made in most instances after descriptions alone are open to criticism. The arrangement of the descriptions of the fungi has been made according to the classification adopted by Lindau in Rabenhorst's "Kryptogamen Flora" (68).

The author is here taking the privilege of expressing his sincere thanks to Dr. Charles Thom, of the Bureau of Chemistry, U. S. Department of Agriculture, for the identification of several of the Penicillia; to Dr. A. F. Blakeslee, of Cold Spring Harbor, for the determination of the sexual reaction of the Mucors, and determination of several Mucorales; to Dr. A. H. Chivers, of Dartmouth College, for the identification of the Chaetomia; to Dr. C. D. Sherbakoff, of the Florida Experiment Station, for the identification of the Fusaria; to Mrs. F. W. Patterson, of the Bureau of Plant Industry, U. S. Department of Agriculture, for the examination of several organisms; to Dr. M. T. Cook and Mr. C. A. Schwarze, of the New Jersey Agricultural Experiment Station, for different suggestions in the course of the work; and particularly to Dr. J. G. Lipman, Director of the New Jersey Agricultural Experiment Station, for the many helpful suggestions in the outlining of the work.

Phycomycetes

Mucorales

Absidia

Absidia Lichtheimi (Lucet et Constantin) Lendner (A. 30)

This organism forms a white floccose growth upon the No. II medium. The sporangiophores are creeping, terminated by elongated branches, carrying sporangia. Sporangia hyaline, pear-shaped, with an apophysis gradually passing into the stalk of the sporangiophore; it is 22 to 48 μ in diameter, with a smooth membrane, which dissolves easily, leaving a small collarette; columella globose to hemispherical, 14 to 21 μ in diameter, usually smooth or with a short central spine. Spores globose to oval, hyaline, 4 to 6.8 μ in diameter.

Hab. Isolated once from garden soil; also by Lendner in Switzerland.

Absidia Orchidis (Vuill.) Hagem (G. 24)

Colony dense, brown, 2 to 3 cm. high. Stolons very strongly branched. Sporangiophores are 65 to 250 μ high by 6.2 to 8.8 μ thick, usually in groups of two, often as many as six, always with a septum below the sporangium. Sporangia 17 to 50 μ high and broad, without the apophysis; membrane incrusted with fine granules; the membrane dissolves leaving a rigid collar. Columella conical or hemispherical, spiny or smooth, 10 to 22 μ high and broad. Spores spherical to slightly elliptical, 2.3 to 3.2 μ in diameter, hyaline singly, but weak-yellow in mass (Pl. I, fig. 1-3).

Hab. Isolated from iron soil; also by Hagem from Norway soils.

Mucor

This group of organisms forms together with the Penicillia and Aspergilli the three dominant groups of soil fungi in total numbers and in variety of species. One is most likely to find this group in greater abundance in the soil, than any other group of soil organisms, because the Mucors grow readily on most of the media used for the isolation of soil forms, and will easily overrun the other organisms. But if media are used, such as Czapek's agar, which will not favor the rapid development of the Mucorales, the Penicillia will be found to be even more widely spread in the soil than the Mucors. As to types and numbers in the soil, this group is very important. Hagem (42, 43, 44) did a great deal of work on the Mucors of the soil in Norway. He divided them into classes as to their common associative occurrence. It is characteristic that the *Mucor Ramannianus* group, found by Hagem to be one of the most common groups of soil Mucors, has been isolated by the writer only to a very small extent. *Mucor Ramannianus* has not been isolated at all and *Mucor silvaticus* was isolated only once from the orchard soil.

The common groups of Mucors in the soils studied were found to be the *Mucor circenelloides, Mucor plumbeus, Mucor racemosus,* and *Zygorhynchus Vuilleminii.* These four groups have been found in most of the soils studied, at every isolation; and it seems peculiar that these four organisms, or groups of organisms, seem to be among the very few which lead an active life in the soil, as was shown by the direct inoculation method.

All the Mucorales have been studied on the No. II medium.

Mucor hiemalis Wehmer

Colony at first gray, later becoming brownish, 1 to 3 cm. high. Sporangiophores at first erect, later bending down, unbranched in young cultures, with some branching after culture is 10 to 12 days old. Sporangia yellow-brown with a tinge of green, globose, 52 to 106 μ in diameter; sporangial wall dissolving, leaving a small collarette. Columella globose to somewhat oval, 32 to 52 μ high and 23 to 40 μ broad. Spores elliptical, 4.5 to 8 x 3 to 5.2 μ. Chalmydospores numerous, globose to barrel shaped, 10 to 14 μ in diameter, occurring sometimes in the sporangiophores (Pl. II, fig. 7-9).

Hab. Isolated several times from the garden, orchard and forest soils; by Hagem in Norway, Lendner in Switzerland, Namyslowski in Austria, Jensen in Ithaca, and McLean and Wilson in New Jersey.

Mucor microsporus Namys. (B. 12)

The general characters of this organism, when grown of the No. II medium, coincide very closely with those given by Namyslowski (77).

Hab. Isolated once from the orchard soil; by Namyslowski in Austria, and McLean and Wilson in New Jersey.

Mucor circinelloides van Tieghem (C. 29)

Colony at first gray, later becoming gray-brown to brown, 1 to 1.5 cm. high. Sporangiophores, up to 1 cm. high, branched sympodially, usually with regular branching to right and left; side branches are slightly bent, all ending in sporangia. Sporangia spherical, gray-brown when mature, 35 to 67 μ in diameter; wall dissolving easily in the case of the lower, larger sporangia, but the upper, smaller ones, often remain intact; wall is incrusted with fine grains, and leaves a basal collar, when dissolving. Columella globose to half-globose, colorless, smooth, 14 to 35 μ in diameter. Spores globose to elliptical, 3.5 to 5.2 x 3.2 to 3.8 μ. Chlamydospores intercalary, hyaline, smooth, spherical to oval, 10 to 19 μ in diameter. This organism was found by Dr. Blakeslee to give a (—) or male reaction (Pl. II, fig. 4-6).

Hab. Found to be one of the most common species in the soils examined. It has been isolated about 100 times from all the local soils, also from the Oregon and California soils. The wide distribution of this organism is also associated with its life-activity in the soil; also by Hagem in Norway, Dale in England, and Jensen in Ithaca.

Mucor plumbeus Bonorden

Syn.: *Mucor spinosus* van Tieghem

This organism, as well as the *Mucor circinelloides*, forms a very common and widely distributed soil organism. But it varies greatly in size and shape of columella and spores, so, the author believes, that this is not one single species, but it embraces a group of organisms, having a spiny columella. This fact seemed to have been recognized already by Lendner (66), who formed a new species—*Mucor spinesceus.* A similar idea concerning this organism has been expressed by Dr. Blakeslee. Colony black-brown, up to 2 cm. high. Sporangiophores 7 to 10 μ in diameter, upright, rigid, mixed monopodially and cymose-symbodially branched, each branch ending in a sporangium and having a septum at the base. Sporangia brown, spherical, with dissolving wall. No sexual reaction was found in any of the strains (Pl. II, fig. 10-13).

Strain I (D. 23). Sporangia 40 to 60 μ in diameter. Columella short cylindrical, brown, with a basal collar, having 1 to 3 spines 16 to 35 x 10 to 38 μ, or none at all. Spores globose to somewhat elliptical, 5.2 to 9.2 μ in diameter, yellowish when single, brown in mass.

Strain II (C. 22). Sporangia and columella are quite variable in size. Sporangia dark brown, 42 to 86 μ in diameter. Columella oval, subcylindrical, and cylindrical, 20 to 32 x 14 to 19 μ, bearing a variable number of spines. Spores spherical, 7 to 8 μ in diameter. Chlamydospores cylindrical to egg-shaped, 7 x 14 μ.

Strain III (C. 20). This organism closely approaches Lendner's form *Mucor spinesceus.* Low gray growth, 1 to 2 mm. high, becoming dark to almost black with age. Sporangiophores rigid, sometimes slightly curved. Sporangia globose,

18 to 60 μ in diameter, having a somewhat spiny membrane which dissolves, leaving a basal collar. Columella globose to cylindrical, oval or pyriform, spiny, 7 to 18 x 10 to 35 μ. Spores globose to oval, 4 to 6 x 5 to 7 μ, yellowish in color. Chlamydospores spherical to barrel-shaped.

Hab. This organism, or rather group of organisms, has been isolated from the garden, orchard, meadow, and California soils; also by Hagem in Norway, Jensen in Ithaca, and Dale in England.

Mucor racemosus Fres. (A. 31)

Colony gray at first, later becoming brown, 1 to 2 cm. high. Sporangiophores varying greatly in length, 15 to 24 μ thick, unbranched when young, then branched monopodially with numerous chlamydospores, singly or in chains, spherical, angular or cylindrical, set free when ripe. Sporangia greatly varying in size, globose, yellow-brown, with a smooth, non-dissolving membrane, 70 to 140 μ in diameter. Columella oval to egg-shaped, somewhat cylindrical at the base, always higher than broad, 38 to 65 x 52 to 140 μ. Spores oval, 3 to 6 x 6 to 9 μ.

This organism gave no sexual reaction.

This species should be looked upon also as a group of organisms rather than as a single species, for the abundance and great variability of the different strains, especially when isolated from different soils.

Hab. Isolated from garden, orchard, meadow, and Oregon soils; also by Adametz in Germany, Lendner in Switzerland, Hagem in Norway, Dale in England, Jensen in Long Island, and McLean and Wilson in New Jersey.

Mucor silvaticus (?) Hagem (B. 15)

Colony at first white, then gray. Sporangiophores 1 to 2 cm. high, branching sympodially, the stalk being separated from the further growing branches by a septum. Sporangia never sessile, light brown, globose, 40 to 60 μ in diameter, with smooth, easily dissolving wall. Columella globose, hyaline, 12 to 38 μ in diameter, with a basal collar. Spores elliptical to cylindrical, 2.3 to 2.8 x 3.5 to 5.2 μ. Chlamydospores lemon-shaped, yellowish, 14 x 18 μ. Oil drops found abundantly in hyphae.

This organism gave a (+) or female reaction.

Hab. Isolated once from orchard soil; also by Hagem in Norway.

Mucor botryoides Lendner

Colony at first light brown, later becoming grayish-brown, consisting of a cobweb-like network, up to 2 cm. high. Sporangiophores bending, 15 to 18 μ in diameter, ending in a large sporangium, with a cluster of branches below, each terminated by a sporangium. Sporangia globose, at first gray, then turning yellow-brown, with dissolving membrane; terminal sporangium up to 75 μ in diameter. Columella oblong, 12 to 35 x 17 to 52 μ, hyaline. Spores globose, smooth, 5.2 to 9.8 μ in diameter (Pl. I, fig. 4, 5).

Hab. Isolated several times from Sassafras loam; also by Lendner in Switzerland, Jensen in Ithaca, and McLean and Wilson in New Jersey.

Mucor saturninus (?) Hagem (B. 6)

Colonies at first lead-gray, later becoming black. Sporangiophores varying in height from 1 mm. to 2 cm., branched monopodially and sympodially. Sporangia finally becoming black, 25 to 150 μ in diameter. Columella oval, globose, to egg-shaped, 10 to 52 x 15 to 68 μ. Spores spherical to somewhat elliptical, 4 to 5 x 4 to 8 μ in diameter (Pl. I, fig. 6-8).

Hab. Isolated from the orchard soil; also by Hagem in Norway.

Mucor sphaerosporus Hagem (B. 22)

Colony yellow-brown with a grayish tinge. Sporangiophores differing greatly in height, 0.2 to 1.2 cm., the longer ones usually sympodially branched. Sporangia globose, yellow-brown, 35 to 85 μ in diameter; sporangial wall encrusted with fine grains and breaking up into small fragments, which cling to the spore masses. Columella oval, slightly attenuated at the base, 25 to 58 x 27 to 67 μ. Spores spherical, smooth, 5.2 to 9.8 μ in diameter. Chlamydospores abundant, occurring both on the vegetative hyphae and sporangiophores. Reaction of culture is (—) or male.

The culture of this organism agrees closely with the one received from Miss Dale, but the abundant large spores were not found in it.

Hab. Isolated from the orchard soil; also by Lendner in Switzerland, Hagem in Norway, and Dale in England.

Mucor sp. (C. 44)

Colonies low and slow growing, gray-black to black. Sporangiophores 200 to 3000 μ high by 8 to 14 μ thick, septate, branched sympodially and monopodially, some branches being very short and carrying circinate sporangia. Sporangia globose, black, 30 to 90 μ in diameter, wall fracturing and leaving a basal collar. Columella hyaline, variable in size, usually egg-shaped to spherical, 10 to 45 x 15 to 52 μ. Spores spherical, gray, 3.5 to 4.4 μ in diameter (Pl. I, fig. 10, 11).

Hab. Isolated once from the meadow soil.

Mucor sp. (D. 28)

Colony forms at first a solid gray growth, which becomes later yellowish-brown to dark gray; hyphae creeping, filled with oil globules, .6 to 8 μ thick. The sporangiophores form loose tufts, up to 1.5 cm. high, 6 to 35 μ thick, monopodially branched, with numerous chlamydospores, and rough surface. Sporangia 40 to 170 μ in diameter, yellow in color, later becoming black; wall breaking, leaving a small collar at the base of columella. Columella oval, higher than broad, and broader at the base than at the top, 20 to 45 x 25 to 88 μ. Spores usually egg-shaped, large, 5 to 8 x 6 to 12 μ, of a yellowish color. Chlamydospores very abundant, yellow, spherical, egg-shaped, or cylindrical, 17 to 26 μ in diameter (Pl. I, fig. 12-14).

Hab. Isolated once from the forest soil.

Mucor glomerula (Bainier) Lendner (C. 4)

This organism coincides very closely to (C. 8) received from Miss Dale. Colony brownish-white, up to 3 cm. high. Sporangiophores, 13 μ in diameter, form a cluster of short branches, or end in a terminal sporangium, surrounded by a verticil of secondary sporangia. Sporangia spherical, dark brown, with fine hairs on the surface, 25 to 72 μ in diameter; wall dissolving, leaving a basal collar. Columella smooth, flattened or subcylindrical, 16.4 to 18 x 21 to 28 μ or 44 x 36 μ when subcylindrical. Spores smooth, globose, 3.2 to 5.6 μ in diameter. Chlamydospores spherical to elliptical, 4.6 to 5.4 x 4.8 to 8.8 μ. The formation of branching, rhizoid-like sporangiophores in contact with the glass of the tube is very characteristic (Pl. II, fig. 1-3).

Hab. Isolated from meadow and iron soils; also by Lendner in Switzerland, and Dale in England.

Mucor flavus Banier (G. 2)

Colony at first white, later becoming gray, up to 2 cm. high. Mycelium is filled with large oil globules. Sporangiophores branched monopodially, yellowish color,

12 to 30 μ thick; sporangia yellow, 48 to 205 μ in diameter, with easily dissolving wall. Columella ovate, 37 to 52 μ broad, 41 to 95 μ high; spores elliptical to spindle-shaped, hyaline, 2.7 to 4.4 x 5.3 to 9.6 μ.

Hab. Isolated from iron soil; also by Hagem in Norway, and Lendner in Switzerland.

Zygorhynchus

Zygorhynchus Vuilleminii Namys.

This organism is found to be one of the most common soil fungi, having been isolated from all the soils studied. It is very easily isolated, when a sample of subsoil, 12 to 20 inches deep, is inoculated directly into a plate of sterile medium. This organism will be one of the very few developing out of the soil upon direct inoculation.

CZAPEK'S AGAR. A creeping, much branching mycelium, without any surface growth, except the sporangial masses.

MEDIUM No. II. White floccose growth, becoming gray, then black from the mass of zygospores. Sporangiophores hyaline, smooth, pendant or upright, septate, 100 μ long and 8 to 10 μ thick. Sporangia spherical, 30 to 90 μ in diameter, yellow to waxy-brown, with a fine, spiny membrane. Wall dissolving and leaving a basal collar. Columella spherical to somewhat flattened, 22 to 28 (12 to 43) μ in diamcter. Spores elliptical to cylindrical, 1 to 2.5 x 4 to 4.4 μ, size varying greatly with strain isolated. Chlamydospores single or in chains, globose, egg-shaped, or cylindrical, 12 to 18 x 15 to 22 μ. Zygospores formed in large numbers, 21 to 43 μ in diameter, of a deep brown color and covered with short tubercules (Pl. III, fig. 1-5).

Dr. Blakeslee identified this organism as *Zygorhynchus Moelleri* Vuill., closely related to *Zygorhynchus Vuilleminii* Namyslowski.

Hab. Isolated from all the soils examined by the writer; also by Namyslowski in Austria, Hagem in Norway, McLean and Wilson in New Jersey, and Jensen in Long Island.

Rhizopus

Rhizopus nigricans Ehrenberg

This organism agrees closely with the one received from Dr. Blakeslee.

Hab. Isolated from garden, orchard, meadow, and Oregon soils also by Adametz in Germany, Hagem in Norway, Jensen in Ithaca, and McLean and Wilson in New Jersey.

Rhizopus nodosus Namys. (B. 5)

Identified by Dr. Blakeslee.

Hab. Isolated from orchard and meadow soils; also by Lendner in Switzerland, and Hagem in Norway.

Rhizopus sp. (B. 3)

Several more species of Rhizopus have been isolated from the orchard and other soils. No attempt was made to identify them, for the lack of information on this genus, as well as the lack of time to carry on extensive biochemical investigations, which would help to distinguish them. The work of Hanzawa (46) is a step forward in the study of this genus. The form (B. 3) was found by Dr. Blakeslee to be neutral, with no sexual reaction.

Ascomycetes

Saccharomyces sp.

Several species of yeasts have been isolated from the different soils, including yellow, orange, and white species. But no effort was made to identify them.

Chaetomium olivaceum Cooke and Ellis

The description of this organism closely agrees with the one given by Jensen (53).

Hab. Isolated once from meadow soil; also by Jensen in Ithaca.

Chaetomium cochliodes Palliser

This organism has been identified by Dr. Chivers.

Hab. Isolated once from Sassafras loam.

Fungi Imperfecti

HYPHOMYCETES

Mucedenaceae

Monilia

Monilia sitophila (Mont.) Saccardo (C. 25)

Colony white, floccose, spreading. Red masses of conidia appearing on the margin, usually at the rim of the Petri dish; the growth on a slant is far above the substratum, close to the glass of the tube. Vegetative hyphae hyaline, greatly branched, septate; surface mycelium carrying short branches, from which the numerous conidia are formed. Conidia egg-shaped to cylindrical, 5.2 to 13.4 μ in diameter; they are formed by the rounding off of the cells separated by partitions from the mother cell; conidia give rise to new ones by budding.

Hab. Isolated from garden and meadow soils; also by McLean and Wilson in New Jersey.

Monilia humicola (?) Oudemans (C. 42)

Colony consists of loosely interwoven hyphae, colorless when young, green when old, creeping, branched, septate, 4.8 to 5.2 μ in diameter. Fruiting hyphae are produced as alternate or opposite side branches of the vegetative hyphae and are closely septate. Conidia yellow to greenish, smooth, elliptical, 2.4 to 3.6 x 2.7 to 5.8 μ (Pl. III, fig. 6).

Hab. Isolated once from the meadow soil; also by Koning in Holland.

Oidium

Oidium lactis Fres. (A. 22)

Growth forms a white, slimy cover over the medium. Mycelium consists of irregularly branching, septate hyphae; conidia are formed by the separation of the oidial bodies by partition walls. The whole hypha may break down into single cells, which are not rounded at the ends. Chains of oidia often form a zigzag line, because the individual spores still stick to one another; spores 4.4 to 6. 4 x 6.2 to 8.8 μ. Chlamydospores are also found (Pl. IV, fig. 14).

Hab. Isolated several times from garden, orchard, and meadow soils.

Oidium sp. (A. 30)

Colony white floccose, up to 2 cm. high; at the base of the growth, where it starts from the substratum, it is of a dirty green color, later becoming brown;

reverse of colony is dark green, substratum becoming colored brown. Mycelium consists of irregularly branching hyphae, which break up easily into cylindrical cells, with edges only slightly rounded at the corners. Cells differ greatly in size, 5.2 to 8.5 x 6 to 35 μ. Contents hyaline or slightly granular. No actual budding was found to take place, only some spores have shown slight swelling on the sides, which reminded one of budding (Pl. IV, fig. 13).

Hab. Isolated once from the garden soil.

Sporotrichum

Sporotrichum sp. (pulviniforme ?) Thüm (G. 6)

Colony white, spreading, cushion-like, with a slightly slimy surface. Hyphae creeping, hyaline, septate, little branched, 2 to 2.5 μ in diameter. Conidiophores arising as short side branches up to 20 μ long. Conidia formed in groups on the sides of the hyphae, or on the tips of the branches; elongated, rounded at the ends, 2.2 to 3 x 4 to 6 μ, with two oil drops in each.

Hab. Isolated twice from the iron soil.

Sporotrichum roseum (?) Link

This form coincides very closely with that described by Miss Dale under (D. 5).

Hab. Isolated once from the garden soil; also by Dale in England.

Botrytis

Botrytis cinerea Pers. (C. 28)

Colony gray to gray-green, finally becoming dark to almost black, surface gray from the conidial masses. Mycelium hyaline, branched, septate, 3.5 μ in diameter. Conidiophores erect, septate, branching only at the tip, 11 to 18 μ in diameter. Conidia form a thick head around projections of the top branches; they are soon separated, ovate to elliptical, finely apiculated at the base, 10.2 to 17 x 7 to 8.5 μ, with hyaline membrane.

Hab. Isolated once from meadow soil; also by Lendner in Switzerland.

Aspergillus

This genus is in some soils most representative in numbers, though not found in such a large number of species as the genus Penicillium. Some 18 species have been isolated from the soil, but only 12 of them are described here; the others are still under observation.

Aspergillus fumigatus Fres. (C. 18)

This organism is similar to the one described by Lindau (68).

Hab. Isolated repeatedly from all the local soils, from the Oregon soil; also by Jensen in Ithaca.

Aspergillus nidulans Eidam

Closely coincides with Lindau's (68) description.

Hab. Isolated repeatedly from all the local soils; also by Goddard in Michigan.

Aspergillus diversicolor Vuill.

Colony at first green, later changing to a rosy tinge, and finally the whole colony becomes rose colored, with the production of a soluble red pigment. The green color always appearing first, the rose color developing only later, never to become green again. In some strains, after many transfers, the rose color appeared

at once, without any green coming in its successive stages. Vegetative hyphae, 1.6 to 2.1 μ in diameter, filled with oil globules; fertile hyphae 300 to 500 x 4.4 to 6.2 μ. Swelling spherical to somewhat elongated, 13 to 16 x 17 to 23 μ, closely beset with branching sterigmata; primary sterigmata 4.4 to 9.2 x 3.5 μ; secondary sterigmata 4.6 to 8.8 x 2.4 to 2.8 μ. Spores globose, warty, 2.7 to 3.4 μ in diameter.

Hab. Isolated repeatedly from all the local soils, and also from the iron and California soils.

Aspergillus niger van Tieghem

Mycelium at first white, then becoming canary-yellow. All the forms isolated produce the yellow color in the substratum. Conidiophores up to 2 mm. long and 8.5 to 15 μ in diameter; swelling globose, 35 to 43 μ in diameter. Primary sterigmata 16 to 19 x 4 to 4.5 μ, carrying 3 to 4 secondary sterigmata 8 x 3 μ, each ending in a long chain of spores. Conidia smooth at first, becoming warty with age, brownish when single, deep brown to black in mass; spherical, thick-walled, uniform in size, 3.5 to 3.8 μ in diameter. Sclerotia at first yellow, then becoming brown, 166 to 300 μ in diameter.

The different strains of this organism are quite distinct from one another, the above description being based upon the strain (B. 16), which approaches the general description of *Aspergillus niger* more closely than any other strain; (C. 28) for example, formed spores 4.2 to 4.6 μ in diameter.

Hab. Isolated from garden, orchard, and timothy soils. A common organism in the soil, though not so widely distributed as the first three Aspergilli.

Aspergillus (n. sp. ?) (C. 19)

Colonies of a golden yellow color, with a brown reverse, and no pigment production in the substratum. Sterile hyphae creeping, branched, septate, yellowish in color; margin of colony is large, transparent, and spreading. Conidiophores unbranched, unseptate, arising directly from the substratum or as side branches of the vegetative aerial hyphae, have a tendency to bend slightly right below the fruiting body, 200 to 450 (50 to 1000) μ high by 8.8 to 13 μ thick, terminating in a globose swelling; the surface of the conidiophore is rough and brown in color. Swelling brown in color, 31 to 44 (17.5 to 52) μ in diameter, closely beset with simple radiating sterigmata, 5 to 17 x 3.2 to 3.6 μ; together with the simple sterigmata, branching ones occur; the primary sterigmata are then 13.2 x 6.2 μ, secondary 7.2 x 3.5 μ. Conidia yellowish when single, red-brown in mass, globose, 3.5 to 4.8 μ in diameter, and forming long chains. Germination of the conidia takes place through one tube. Gelatin is liquefied in 15 days at 18° C. Chlamydospores present in the vegetative hyphae, 4.4 to 10.2 μ in diameter (Pl. III, fig. 7, 8).

Hab. Isolated several times from timothy soil.

Aspergillus calyptratus (?) Oud. (G. 15)

Colony red-brown, with a yellowish spreading margin; reverse brown, slightly coloring the medium. Vegetative hyphae hyaline, septate, 1.8 to 2.3 μ thick; conidiophores arising directly from the substratum or as side branches of the vegetative hyphae, smooth, hyaline, septate, 42 to 170 μ high and 4.4 to 5 μ thick. Sterigmata simple, pointing forward, very close, numerous, cylindrical, 2.1 to 2.6 x 5 to 7.2 μ. Conidia globose, 2.4 to 2.8 μ in diameter, smooth, vacuolated, hyaline singly, brown in mass, born in long chains and forming a dark cylindrical head, 42 to 56 x 80 to 170 (40 to 83 x 80 to 425) μ. Swelling ellipsoidal to obpyriform, 7.1 to 13.2 x 12 to 18 μ. Chains of spores not broken up readily when mounted (Pl. IV, fig. 1, 2).

Hab. Isolated from iron soil; also by Koning in Holland.

Aspergillus fuscus Schiemann (A. 29)

This organism agrees very closely with the culture received from Dr. Thom.

Hab. Isolated from garden soil.

Aspergillus flavus Link (N. 41)

Colonies bright green, at first light, then darker, edge of colony yellowish brown. Vegetative hyphae hyaline, 2.2 to 2.7 μ in diameter; conidiophores 500 to 1500 μ high by 8.5 to 17 μ thick, surface rough, colorless. Swelling spherical, 26 to 31 μ in diameter, with a rough surface and yellow-green contents. Conidial head up to 400 μ in diameter. Sterigmata unbranched, radiating, 3 to 3.6 x 12 to 15 μ. Conidia bright green, globose, 3.2 to 5.2 μ in diameter. Long chains of spores not broken easily when mounted. Perithecia not observed. This culture compares favorably with the one received from Dr. Thom, as the Amsterdam strain.

Hab. Isolated from California soil.

Aspergillus sp. (N. 40)

Colony snow-white, spreading, reverse creamy; there is a scant production of conidial fructifications and an abundant formation of sclerotia, produced in large numbers when culture is about 8 to 10 days old. Conidiophores 250 to 300 (165 to 1100) μ long by 5.4 to 10.6 μ thick, arising directly from the substratum or as side branches of aerial hyphae; surface rough, yellowish. Sterigmata pointing forward, forming a solid head, 42 to 175 μ long by 80 to 100 μ thick, thinner at the base than at the top. Primary sterigmata 1.8 to 2.6 x 7.1 to 10.6 μ, secondary 2.2 to 2.7 x 5.4 to 7.8 μ. Conidia yellowish, spherical, 2.2 to 3.4 μ in diameter. Swelling globose to oblong, 10 to 30 x 18 to 35 μ. Sclerotia spherical and subcylindrical, yellow in color, 125 to 800 x 165 to 1000 μ.

Hab. Isolated from California soil.

Aspergillus clavatus Desmasières

Colony gray-green, surface powdery; reverse of colony brownish. Vegetative hyphae hyaline, 2.1 to 2.8 μ thick; conidiophores 400 to 3000 μ high by 21 to 26 μ thick, marginal ones shorter and white, while central ones are tall and lead-gray. Fruiting bodies at first elongated, 65 to 132 x 125 to 300 μ, later becoming more rounded; swelling club-shaped, 35 to 62 x 200 to 350 μ, surrounded by simple, radiating sterigmata, 2.6 x 4.6 to 8.8 μ; conidia oval-shaped, 2.1 to 2.8 x 2.7 to 3.6 μ. By selection the author has been able to isolate two strains from the original, differing only in the length of conidiophores, one having average length of conidiophores over two times as long as the other.

Culture agrees in all respects with the one received from Dr. Thom.

Hab. Isolated once from Sassafras loam.

Aspergillus repens de Bary

The general characters of this organism are exactly the same as for *Aspergillus glaucus*, but all the parts are smaller in size; this point places it with the *"repens."* Colony at first light green, changing to dark green, and soon becomes covered with golden-yellow perithecia. Conidiophores 85 to 250 μ long by 8.8 μ thick. Swelling spherical, 22 to 31 μ, carrying unbranched sterigmata, 3.6 x 7.2 μ. Conidia green, globose to egg-shaped, spiny, 3.5 to 5.3 x 3.5 to 6.2 μ. Perithecia produced in abundance, yellow in color, spherical to elliptical, with an uneven surface, 85 to 150 μ in diameter; asci spherical to ovate, 8.8 to 14 μ in diameter, containing 5 to 8 smooth, elliptical spores, 4.8 to 5.6 x 5.6 to 6.4 μ (Pl. IV, fig. 3-6).

Hab. Isolated once from forest soil.

Penicillium

This genus forms one of the largest groups of soil fungi; more species have been found in the soil belonging to this genus than to any other one. Some forms have been often found to form 2 to 3 colonies on a plate of 20,000 to 200,000 dilution. Very often plates of 20,000 dilution would contain 15 to 30 colonies of Penicillia, usually of one to four species. The writer has not been able to get many Penicillia from the direct soil inoculations, but from this it should not be concluded that these organisms are merely temporary visitors in the soil, and do not grow there and produce spores. If that should be the case, one would hardly expect to get Penicillia spores at a depth of 20 to 30 inches, because they would not be carried down by the water so easily, since they would germinate in the process of being carried down. The large numbers of spores found and the fact that they are found in all soils examined, cultivated and uncultivated, justify also the conclusion that the Penicillia are not merely brought in temporarily by some outside agency, but probably live in the soil. Oudemans (80) found 5 species of Penicillia in the soil, Jensen (53) also found 5, Dale (27) found 17 species of Penicillia and 3 species of Scopulariopsis in all the different types of soil that she has examined. The writer isolated about 35 well defined species and groups. Only about 25 of them are described here. A great deal of assistance in the identification of the Penicillia has been given to the writer by Dr. Charles Thom. Drawings and more complete description of the unidentified forms will appear later.

Luteum-purpurogenum group Thom

This is a typical group of soil organisms; more forms were found in the soil belonging to this group than to any other one; representatives have been isolated from all soils at one time or another. Several well-defined species belonging to this group have been isolated: *Penicillium luteum* Tukal, from the garden soil; *Penicillium pinophilum* (Hedgcock) Thom, forest soil; *Penicillium lilacinum* Thom, meadow soil; and *Penicillium purpurogenum*, var. *rubri sclerotium* Thom, meadow soil. Besides these, several forms have been isolated, belonging to this group, but which could not be placed with any well-described species; most of them were nearer the "luteum" end of the group. The differences were due only to the shade and change in color.

Penicillium chrysogenum Thom

Corresponds closely with Thom's description. One strain was isolated, which proved to be the same as Thom's No. 57; it produces an orange color on the reverse without coloring the medium.

Hab. Isolated from timothy, Sassafras, and California soils.

Penicillium commune Thom

Agrees closely to culture received from Dr. Thom.

Hab. Isolated once from orchard soil.

Penicillium decumbans Thom

Hab. Isolated from forest soil.

Penicillium digitatum Sacc.

Hab. Isolated from garden, forest, and iron soils.

Penicillium expansum (Link) Thom

Hab. Isolated from forest soil.

Penicillium italicum Wehmer

Hab. Isolated from California soil.

Penicillium oxalicum Thom (A. 44)

Hab. Isolated once from garden soil.

Penicillium notatum Westling (C. 2)

Colony at first white, subfloccose, later becoming green, with a yellow reverse. Gelatin is liquefied in 7 to 8 days. Calcium oxalate crystals are formed abundantly on raisin agar. Conidiophores arising from the substratum, or as side branches of the aerial mycelium, smooth, up to 800 μ long and 2.8 to 5 μ thick, branching. Metulae 3 to 4.5 x 9.2 to 14 μ; sterigmata 2.4 to 3 x 7 to 8.4 μ. Conidia spherical, smooth, 2.6 to 3.3 μ in diameter. Closely agrees with culture received from Dr. Thom.

Hab. Isolated from garden, meadow, and forest soils.

Penicillium viridicatum Westling (A. 9)

Colony at first light green, slightly elevated above the substratum, restricted in growth; reverse yellow to brown, with the production of a yellow-brown pigment in the substratum. When old, surface becomes deep dull yellow-green (Rdg. xxxii, 31"-k) (88), granular, with the exudation of yellow drops of water upon the surface. Conidiophores, 50 to 500 μ long by 4 to 6 μ thick, arising as side branches of the aerial hyphae, verticillately branched, and ending in loose columns of spores; metulae 4 to 6 x 10 to 14 μ; sterigmata 3 to 3.5 x 8 to 9.5 μ. Conidia smooth, hyaline, globose, 2.6 to 4.4 x 3.3 to 4.4 μ. Liquefaction of the gelatin is rapid, starting at 5 days, at 18° C.

Hab. Isolated from garden, orchard, and iron soils.

Penicillium atramentosum Thom

Hab. Isolated from garden soil.

Penicillium rugulosum Thom

Hab. Isolated from forest soil.

Penicillium cyclopium Westling (D. 33)

Hab. Isolated once from forest soil.

Penicillium lividum Westling (D. 5)

Colonies at first white, with a green center and spreading margin, turning later gray-green and finaly becoming light Danube green (Rdg. xxxii, 35" k), with a white margin. Reverse yellow to yellow-brown, with the production of a soluble yellow pigment. No liquefaction of the gelatin in 15 days. Conidiophores arising

as side branches of the aerial hyphae, 42 to 325 μ long by 2.4 to 3.6 μ thick, unbranched, ending in a verticillate head; sterigmata numerous, 2 to 2.5 x 8 to 12 μ. The verticills bear long chains of conidia, 120 to 750 μ long and forming close columns. Conidia greenish, elliptical to egg-shaped, or globose, granular, 3 to 4.2 x 3.4 to 4.8 μ. This organism has been identified by Dr. Thom.

Hab. Isolated from forest soil.

Penicillium glaber Wehmer (A. 16)

This organism belongs to the "Citromyces" group, formed by Wehmer. Colony is dark green in color, with a velvety surface; reverse at first ivory, later becoming dark to almost black; medicum gets a dark tinge. Vegetative hyphae 2.1 to 4.4 μ thick; conidiophores upright, septate, each ending in a single verticil of sterigmata. Conidia form long chains, the dense columns being 160 to 180 (80 to 830) μ long. Conidia uniform in size, globose, vacuolate, hyaline singly, green in mass, 2.4 to 3.2 μ in diameter. Sterigmata numerous, 2.4 to 3.2 x 8.8 to 10.5 μ. Gelatin liquefied in 8 days. Identified by Dr. Thom (Pl. IV, fig. 9).

Hab. Isolated many times from garden, orchard, forest, and Oregon soils.

Penicillium Pfefferianus Wehmer (D. 7)

This organism also belongs to the "Citromyces" group of Wehmer. Colony floccose, gray-green, with a whitish-gray tinge. Conidiophores arising as short branches of the aerial hyphae, 17 to 40 μ long by 3 μ thick, ending in a swelling, 4 to 7 μ in diameter, on which the sterigmata are borne; these are 2.6 to 3.5 x 6.2 to 8.8 μ. Conidia smooth, colorless to yellowish in mass, spherical, 2.4 to 2.8 μ in diameter; chains not broken when mounted.

Hab. Isolated from forest and Oregon soils.

Penicillium group I (13-25)

Colonies at first bluish gray-green (Rdg. xlii, 41‴), subfloccose, becoming with age brownish-green. Reverse at first colorless, later becoming hydrangea-pink (Rdg. xxvii, 5″ f), till finally it is red-brown; medium not discolored. Conidiophores usually arising as side branches of the aerial hyphae, septate, 65 to 330 μ long by 3.6 to 4.8 μ thick. Fructifications form loose divergent brooms, 40 to 250 μ long, with 1 or 2 divergent branches, sometimes none at all. Metulae 2.5 to 3.6 x 12 to 13.2 μ, each one bearing 2 to 3 sterigmata, 2.6 to 4.4 x 7.2 to 12 μ. Conidia globose to elliptical, vacuolate, smooth when young, granular when old, 2.6 to 3.5 x 2.6 to 4.4 μ. Gelatin is slowly liquefied, beginning after 14 days.

Hab. Isolated repeatedly from all the soils examined; very common in the soil.

Penicillium group II (6-22-23)

Colonies bright green with a brownish reverse; in older cultures surface turns gray-green to a dark ashy color; reverse becomes red-brown to dark yellow-brown; surface velvety. Conidiophores arise from the substratum or as short side branches of the aerial hyphae, 88 to 200 μ long by 1.8 to 2.6 μ thick, each dividing into several branches; each branch ends in a verticil of 3 to 6 sterigmata, bearing more or less divergent chains. Conidiophores variable in length; sterigmata 2.7 to 3.5 x 7 to 13.2 μ bearing chains of spores 7 to 35 μ long. Conidia spherical, hyaline when young, brownish when old, thick-walled and spiny, 2.7 to 3.5 μ in diameter. Under certain conditions of moisture, the spores may be massed together into a ball, the chains instead of remaining distinct break down and form a gliocladium. Gelatin does not begin to liquefy until 4 weeks old. This organism greatly resembles Miss Dale's (C. 3).

Hab. Isolated from meadow, forest, and Oregon soils.

Penicillium group III (9-24)

Colony floccose, at first white, with the production of a pink color in the reverse, later becoming gray to dirty yellow, with a deep red color in reverse, substratum becoming yellowish. On raisin agar the colony is sulphur-yellow with no pink pigment produced. Vegetative hyphae creeping, branched, septate, 2 to 3 μ in diameter, often forming ropes of hyphae. Conidiophores arising alternately as side branches of aerial hyphae, 38 to 1000 μ long by 2.7 to 3.5 μ thick, septate; the nodes are characteristically swollen up to 5 μ in thickness. Conidiophores forming 2 to 3 metulae, pointed at both ends, 3.5 to 4.6 x 10 to 25 μ; each metula bears few sterigmata, 2.3 to 2.7 x 7.2 to 13.2 μ. Chains of spores are divergent, 12 to 35 μ long, of unequal size, with no close broom formation. Chains of spores unbroken when mounted; conidia smooth, vacuolate, globose and elliptical, often pointed at one end; 2.7 to 3.0 x 2.6 to 3.5 μ. No liquefaction of the gelatin.

Hab. Isolated from meadow and forest soils.

Penicillium group IV (2-11-15)

Colonies floccose, white to gray, with an intermixture of yellow; when old the color becomes darker, with a violet tinge; reverse colorless, then creamy, finally dark. Conidiophores arising as alternate side branches of the vegetative hyphae, 25 to 85 μ long by 3 μ thick, septate, unbranched, ending in a verticil of 2 to 3 metulae, 4 x 15 to 22 μ; each metula carries 1 to 3 sterigmata, 3.5 to 5.2 x 5.2 to 12 μ. Conidia form short chains, unbroken when mounted, globose to somewhat ellipsoidal, 2 to 2.6 x 2.2 to 3.4 μ. Liquefaction of the gelatin starting only in 14 days and advancing very slowly.

Hab. Isolated from orchard, timothy and Sassafras loam soils.

Penicillium group V (18-20)

Colony at first pale blue (Rdg. viii, 45 f), later changing to greenish-glaucous-blue (Rdg. xlii, 41''' C); reverse is yellow-brown to ivory, slightly coloring the medium yellow. Surface of colony velvety. Conidiophores either terminal or arising as side branches of the aerial hyphae, 85 to 215 μ long by 2.7 to 3.5 μ thick. Conidial fructifications, up to 250 μ long, consist of simple verticils of sterigmata, bearing divergent chains of spores. Metulae 3 x 8.8 to 15.2 μ; sterigmata 2.4 to 3.2 x 7.9 to 10.2 μ. Conidia globose, hyaline, smooth, 2.7 to 3.6 μ in diameter. Liquefaction of the gelatin is very rapid, starting at 5 days, and is almost complete in 10 days.

Hab. Isolated from orchard and forest soils.

Penicillium group VI (10)

Colony at first pale green to dull glaucous-blue (Rdg. xlii, 41''' f), later becoming chocolate-brown; reverse creamy, medium clear; surface velvety. Conidiophores arising directly from substratum, 330 to 500 μ long by 2 to 3.5 μ thick, and ending in a verticil of sterigmata, carrying a side branch or none at all. Metulae 2.8 x 5.3 to 13.2 μ, sterigmata 2.8 x 8.4 to 11 μ; chains of spores greatly divergent. Conidia spherical to elliptical, hyaline, smooth, 2.4 to 2.8 x 2.4 to 3 μ. Liquefaction of the gelatin is very slow, starting when culture is only 18 days old.

Hab. Isolated from garden and iron soils.

Penicillium desiscens Oud. (C. 15)

This organism resembles Jensen's description more closely than Oudeman's. Colony velvety, dark yellowish-green (Rdg. xviii, 33' m), constricted in growth; reverse pale yellow without coloring the substratum. Conidiophores hyaline, septate,

165 to 600 μ high by 3.5 to 5.2 μ in diameter, very seldom forming side branches, but mostly branching verticillately. Conidiophores ending in a verticil of 3 to 6 metulae, 3.1 to 4.4 x 10 to 13.6 μ, each metula producing 4 to 6 sterigmata, 3.5 to 4.4 x 5.2 to 8.2 μ. Conidial fructification is constricted at the base, forming a more or less close broom, 80 to 500 μ long by 25 to 80 μ wide. Conidia light green, ellipsoidal, 2.2 to 2.8 x 2.6 to 3.5 μ (Pl. IV, fig. 10-12).

Hab. Isolated once from meadow soil.

Scopulariopsis

Scopulariopsis brevicaule Saccardo (n. var. ?) (A. 27)

Colonies gray, turning yellowish-brown to chocolate color; surface velvety, margin broadly spreading. Gelatin rapidly liquefied in 5 days with the production of a strong ammoniacal odor. Conidiophores short, 16 to 42 μ high by 4 to 4.4 μ thick, usually borne as lateral branches of the aerial hyphae, sparingly branching or forming simple chains of conidia. Sterigmata tapering at the end, 3.8 to 4.3 x 13 to 17.2 μ. Conidia light brown in mass, globose to somewhat pear-shaped, with a thick spiny wall, 6.2 to 8.8 μ in diameter (Pl. IV, fig. 7, 8).

Hab. Isolated from garden and iron soils.

Cephalosporium

Cephalosporium acremonium Corda (C. 51)

Colonies snow-white, floccose, with a rose-colored center; later all the colony becomes rose-colored, except the white floccose margin. Vegetative hyphae hyaline, fine, creeping, sparsely septate, branched, 2.2 to 3.1 μ thick. Conidiophores arising as side branches of the vegetative hyphae, unseptate, unbranched, 2.3 to 3.1 x 18 to 44 μ, bearing at the tip a head of conidia; the head is globose, 13 to 20 μ in diameter; conidia numerous, oblong to cylindrical, 1.5 to 2.1 x 4 to 6.6 μ, rose-colored.

Hab. Isolated several times from meadow soil; also by Koning in Holland.

Cephalosporium curtipes (?) Saccardo (C. 35)

Colony orbicular, floccose, white. Vegetative hyphae creeping, branched, septate, hyaline, filled with oil globules, 8.8 to 13.2 μ in diameter. Conidiophores erect, short, unbranched, unseptate, arising as side branches of the vegetative hyphae, 3 to 7 μ long; conidial head globose, 7 to 9.5 μ in diameter; conidia egg-shaped to oblong, filled with minute oil globules, 2.3 to 4 x 4.8 to 13.2 μ.

Hab. Isolated once from meadow soil.

Cephalosporium sp. (G. 23)

Colony floccose, white, later becoming yellowish in color. Mycelium hyaline, creeping, branching dichotomously, sparsely septate, 3.5 to 4.2 μ in diameter; conidiophores arising as side branches of the vegetative hyphae, but are not swollen very much at the tip, 1.8 to 2.5 x 26.4 to 35.2 μ; conidia arising singly at the tip of the conidiophore, pressed to the side by those produced next, they adhere to one another by means of a slime, resulting finally in a head of conidia; heads spherical, greatly variable in size, 17 to 100 μ in diameter, breaking off easily; conidia egg-shaped to elliptical, smooth, granular contents, 2.8 to 4.4 x 5.3 to 9.4 μ (Pl. V, fig. 1, 2).

Hab. Isolated once from iron soil.

Cephalosporium sp. (D. 32)

Colonies dense floccose, white, later becoming green to gray-green, with a white woolly margin. Vegetative hyphae hyaline, creeping, much septate, branched,

4.4 to 7.2 μ in diameter; mycelium has a tendency to form ropes of intermingled hyphae. Conidiophores arising as short side branches of the vegetative hyphae, unseptate, unbranched, pointed at the upper end, 3.8 to 6.4 x 13 to 18 μ; conidia elliptical, bright green singly, of a deeper green in mass, 2.8 to 3.6 x 3.5 to 4.8 μ borne singly, but united together to form a head, 14 to 18 μ in diameter; the head is either borne on the short conidiophores, or directly upon the main hyphae. This species may be closely allied to the Trichodermae, but by the character of the conidiophores and the head it is closely related to the Cephalosporia (Pl. IV, fig. 15).

Hab. Isolated from forest soil.

Cephalosporium n. sp. (?) (C. 56)

Colony thin, white, with rosy conidial masses; growth is hardly noticeable with the naked eye, except when the rosy masses of conidia appear. Hyphae hyaline, septate, creeping, branched, 4.4 μ thick; conidiophores arising as side branches of the vegetative hyphae, somewhat thinner than these, 170 to 350 μ long, branching, often with secondary branches, each branch slightly pointed at the tip and carrying a head of conidia; head 8.5 to 17.5 μ in diameter; conidia colorless, egg-shaped, cylindrical to bean-shaped, greatly varying in size, 2.7 to 3.5 x 4.4 to 8.8 μ (Pl. V, fig. 3-5).

Hab. Directly isolated from the meadow soil.

Trichoderma

This genus, of which only very few species are fully described, occurs commonly in the soil. The two known green forms, *Trichoderma Koningi* and *Trichoderma lignorum*, either one or both of them together, have been isolated repeatedly from the soil by most investigators on soil fungi, from Oudemans (80) to Dale (27). Cook and Taubenhaus (23) have also shown that these organisms cause storage rot of sweet potatoes. The green Trichoderma have been found at one time or another in all the soils examined, especially in the meadow and forest soils, from which these organisms have been isolated repeatedly. In general 5 strains of green Trichoderma have been isolated by the author.

Strain I (C. 16). Colony white, floccose, spreading; green masses begin to appear only when culture is 7 to 8 days old, forming isolated groups, without any zonation. Vegetative hyphae hyaline, septate, branched; conidiophores arising as side branches on the mycelium, septate and branched mostly opposite, each branch carrying a conidial head, 8 to 12 μ in diameter; spores elliptical, 2.2 to 3 x 3.1 to 4.4 μ, hyaline to rare green; chlamydospores abundant, 6.2 x 8 μ. This organism coincides more closely with *Trichoderma Koningi* Oud. than any other one.

Hab. Isolated repeatedly from meadow, forest, iron, and Oregon soils.

Strain II (D. 16). Colony at first white, with fine, hyaline, spreading mycelium. Green conidial masses begin to appear at the center, spreading gradually toward the periphery with the formation of green concentric zones; the zonation is characteristic of this group. Vegetative hyphate creeping, septate; conidiophores do not differ very much from those of the previous organism: there is less septation and greater variation in the size of the conidial head, which is 7 to 17.5 μ in diameter. Conidia globose, green, 3.2 to 3.5 μ in diameter; no chlamydospores were observed. This organism is closely related to *Trichoderma lignorum* (Tode) Harz.

Hab. Isolated repeatedly from meadow and forest soils.

Strain III (G. 5). Colonies at first colorless, thin, rapidly spreading, non-floccose; later becoming light green and changing into a dark green color, with no conidial tufts, as in Strain I, and no zonation as in Strain II. Entire colony soon becomes solid green. Vegetative hyphae hyaline, creeping, septate, and branched. Conidiophores with little branching and carrying fairly large heads of conidia, 17.5 to 35 μ in diameter. Conidia light green, spherical to elliptical, somewhat pointed at one end, 3.5 to 4.4 x 4.4 to 6 μ, largest of all the groups. Chlamydospores abundant, spherical, 8.8 to 13.2 μ in diameter.

Hab. Isolated from iron, garden and forest soils.

Strain IV (C. 10). Colonies at first white, floccose, spreading, with green tufts appearing all over the plate and soon covering the whole surface; the green tufts are more abundant than the vegetative growth, while Strain I had just the opposite relation. Conidiophores arising as side branches of the vegetative hyphae, branched, septate; conidial heads 7 to 10 μ in diameter. Conidia light green, usually elliptical to egg-shaped, but many globose spores are found; conidia 2.7 to 3.2 x 3.1 to 3.6 μ. The large size of the head and the peculiar method of its formation differentiates this organism from the other Trichodermae.

Hab. Isolated from meadow and forest soils.

Strain V (D. 34). Colony very thin, transparent, spreading, with no aerial mycelium except at the very margin. Conidial masses large, with no separate tuft formation, soon covering the whole surface. Vegetative hyphae creeping in substratum, branched, septate. Conidiophores branching, each one ending in a conidial head, 7 to 12 μ in diameter. Conidia elliptical to almost cylindrical, 2.5 to 2.9 x 3 to 3.5 μ, light green singly, dark green in mass; chlamydospores abundant, 5.2 to 8.8 μ in diameter.

Hab. Isolated from forest soil.

Trichoderma album Preuss (C. 17)

Colony thin, white, spreading; small aerial white tufts, 1 to 5 mm. in diameter, appearing on the surface of the colony, without forming regular zones and without covering the whole surface. Vegetative hyphae hyaline, branched, septate; conidiophores arising as side branches of the aerial mycelium, 4 to 4.6 μ in diameter, septate, branching, with small secondary branches; conidial heads 7.5 to 14 μ in diameter; conidia hyaline, elliptical, 1.8 to 2.1 x 2.5 to 2.9 μ.

Hab. Isolated several times from meadow and forest soils.

Acrostalgmus

Acrostalgmus cinnabarimus var *nana* Oud. (A. 3)

The general characters and description of this organism coincide very closely with those given by Oudemans (80).

Hab. Isolated repeatedly from garden, orchard, meadow, and Oregon soils; also by Koning in Holland and Goddard in Michigan.

Acrostalgmus albus Preuss.

Colonies white, thin, subfloccose, spreading rapidly. Vegetative hyphae hyaline, creeping, little branched, septate. Conidiophores arising as side branches of the vegetative hyphae, erect or ascending, up to 200 μ long by 1.5 to 2.1 μ thick, branching at the tip, forming 1 to 6 branches; branches alternate, slightly curved and pointed at the tip. A cap of conidia, 9 to 12 μ in diameter, is borne at the tip of each branch; conidia hyaline, long ellipsoidal, 1.1 to 1.5 x 2.9 to 3.7 μ.

Hab. Isolated from garden and meadow soils.

Verticillium

Verticillium glaucum (?) Bonorden

Colony white, later becoming green from the formation of spores; old cultures are all green, with a yellowish tinge. Mycelium creeping, 3 to 6 μ in diameter; sterigmata arising from conidiophore either singly or in whorls, slightly swollen at the base and pointed at the tip, 3 to 5 x 6 to 10 μ; chlamydospores present; conidia hyaline, spherical, 2.5 to 3 μ in diameter.

Hab. Isolated from forest soil.

Verticillium terrestre (?) (Link) Lindau (G. 16)

Colony snow-white, floccose, spreading, with the production of rose-colored powdery conidial masses. Vegetative hyphae creeping, branched, septate, hyaline; conidiophores upright, septate, branched, with a well-pronounced main stem, 4 to 6.4 μ thick, with four branch whorls; conidia formed singly at the tip of the branches, soon falling off, hyaline, globose to slightly elliptical, 3.8 to 4.5 x 4.4 to 4.8 μ.

Hab. Isolated from forest and iron soils.

Cephalothecium

Cephalothecium roseum Corda

Colony at first white, with a rose tinge, later becoming rose to almost red; growth powdery, spreading, of a cobweb-like nature; vegetative hyphae creeping, septate, branched. Conidiophores unseptate, unbranched, 3 to 3.8 x 200 to 500 μ; conidia produced at the tip of the conidiophore singly, one after another, forming a loose head; conidia pear-shaped, two-celled, rose-colored, 7.6 to 12.4 x 11.4 to 20 μ.

Hab. Isolated from meadow soil; also by Jensen in Ithaca.

Dicoccum

Dicoccum asperum Corda (A. 34)

Colony white, floccose, becoming dark to almost black with a shade of yellow; reverse yellowish black. Mycelium consists of hyaline, branched, sparcly septate, yellowish hyphae, 2.6 to 3.5 μ thick; conidiophores arising as short side branches, 3 to 12 μ long. Conidia at first hyaline, then brown to black, oval, two-celled; upper cell spherical, brown, thick-walled, spiny; lower cell usually smaller, spiny; the whole spore is 20 to 22 μ long, 9 to 13 μ thick.

Hab. Isolated from garden soil.

Zygodesmus

Zygodesmus sp. (B. 36)

Growth floccose, gray, up to 2 cm. high; vegetative hyphae branched, septate, 7 to 10 μ thick. Spores are borne around the tip of the side branches, which vary in length, often almost lacking; the tip of the branch is either not differentiated from the rest or swollen to 27 μ in thickness. The size of the spores varies with the strains; the one isolated from the orchard soil has smooth, yellow, granular spores, spherical to elliptical or egg-shaped, 8.8 to 17.6 x 15 to 22 μ; the Oregon strain has spores of the same shape and color, but smaller in size, being only 5.4 to 8.8 x 6.2 to 10.4 μ; wall either smooth or spiny (Pl. V, fig. 6-9).

Hab. Isolated from orchard and Oregon soils.

Dematiaceae

Basisporum gallarum (?) Molliard (G. 8)

Colony at first white floccose, later becoming dark brown to almost black, with a brownish yellow to black reverse. Mycelium hyaline, branched, septate, creeping,

bearing along its hyphae globose brown to black spores; hyphae filled with oil globules; spores are borne on short lateral branches or directly upon the hyphae; the branches are 4 to 6 x 8 to 12 μ, some of which may consist of two cells; spores spherical, 12 to 15 μ in diameter.

Hab. Isolated from iron soil.

Cladosporium herbarum (Pers.) Link (C. 26)

Syn.: *Hormodendrum cladosporoides* (Fres.) Sacc.

Colony at first white, turning gray to gray-brown, subfloccose; reverse black. Mycelium branched, septate, 2 to 4.6 μ thick; conidiophores much branched, septate, olivaceous in color, 4 to 8.8 x 100 to 200 μ; conidial tufts borne at the end of all the branches. Spores are produced in great abundance, varying greatly in size and shape, always olivaceous, sligbtly elongated and unicellular, 2.2 to 3.8 x 2.8 to 6 μ.

Hab. Isolated repeatedly from garden, orchard, meadow, forest and Oregon soils; also by Jensen in Ithaca, Dale in England, and Goddard in Michigan.

Cladosporium epiphyllum Pers.

Colony at first white, then greenish, and finally gray rusty-brown on the surface and black on the reverse. Conidial masses are borne as side branches of the long radiating hyphae; conidia very numerous, round to cylindrical, at first unicellular, then often with one septum, olive green, 3.8 to 5.2 x 10 to 14 μ (Pl. V. fig. 13).

Hab. Isolated from garden, meadow and California soils; also by Dale in England.

Dematium pollulans (?) de Bary (N. 42)

Colony velvety, dark brown to almost black, reverse black. Hyphae branched, closely septate, brown, 2.8 to 6.2 μ thick. Spores spherical to elliptical, brown, smooth, 3 to 4.4 x 3 to 5.2 μ.

Hab. Isolated once from California soil.

Acremoniella sp. (?) (C. 37)

Colony floccose, spreading, at first white, later turning dark to almost black, with black reverse, aerial mycelium remaining gray. Hyphae creeping, branched, hyaline, with very short side branches, carrying single spores at the tip; spores spherical to egg-shaped, black, 5.2 to 8.8 x 6.2 to 8.8 μ.

Hab. Isolated once from meadow soil.

Alternaria humicola (?) Oudemans (C. 8)

This culture agrees closely with the description given by Oudemans (80).

Hab. Isolated from meadow soil; also by Koning in Holland.

Alternaria sp. (A. 36)

Mycelium branching, hyaline, 3.5 μ thick. Branching chains of spores arising as side branches of the vegetative hyphae. Spores 1 to 4 septate with no cross septa, not or only slightly constricted at the place of septation; spores from almost spherical to cylindrical, 8 to 15 x 15 to 40 μ.

Hab. Isolated from garden and meadow soils.

Alternaria sp. (B. 20)

Colony floccose, dark brown, black on reverse; aerial mycelium gray with a greenish tinge, up to 2 cm. high. Conidia occurring in short chains of 1 to 4 spores;

they are uniform in size, 2 to 4-celled, with one transverse section, 6 to 6.4 x 8.8 to 11.2 μ, slightly or non-constricted at septa.

Hab. Isolated once from orchard soil.

Tuberculariaceae

Fusarium

The author is indebted to Dr. Sherbakoff, of the Florida Experiment Station, for the identification of the Fusaria.

Fusarium angustum Sherb. (I. 5)

Hab. Isolated from Sassfras loam.

Fusarium bullatum Sherb. (F. 1)

Hab. Isolated from an alfalfa soil.

Fusarium solani (Mart. p. var.) Ap. et Wr. (F. 2)

Hab. Isolated from an alfalfa soil. Only the last two organisms have been isolated from the alfalfa soil.

Fusarium orthoceras Ap. et Wr. (B. 17)

Hab. Isolated from garden soil.

Fusarium oxysporium var. *resupinatum* Sherb. (G. 14)

Hab. Isolated from garden and iron soils.

Fusarium caudatum Wr. (D. 19)

Hab. Isolated repeatedly from forest soil.

Fusarium oxysporium Schlech. (G. 21)

Hab. Isolated from iron soil.

In the case of the *F. angustum, F. oxysporium* var. *resupinatum,* and *F. orthoceras,* Dr. Sherbbakoff was not sure of the identification, but this was the nearest he could place them.

Melanconiales

Melanconium sp. (G. 20)

Colony white, subfloccose, soon becoming covered with a green mass of conidia; reverse creamy; the exudation of large drops of water intermixed with the green masses of spores is characteristic. Mycelium thin, branching, filled with oil globules, 1.6 to 1.8 μ thick; spores are borne in dark brown masses, 16 to 28 μ in diameter; spores spindle-shaped, yellowish when single, 2.5 to 2.8 x 6.2 to 8.6 μ.

Hab. Isolated from garden, iron and Oregon soils.

Sphaeropsidales

Coniothyrium Fuckelii (?) Saccardo (C. 28)

Colony at first white, subfloccose, later becoming all black, with white aerial mycelium; reverse at first creamy, later turning black. Hyphae hyaline, branched, septate, anaestomise readily; pycnidia brown to black, seen with the naked eye, 240 to 350 μ in diameter; spores elliptical, slightly epiculate at one end; singly light brown, dark brown in mass, 2.5 to 3.5 x 3 to 5.2 μ (Pl. V, fig. 10-12).

Hab. Isolated once from meadow soil.

Sclerotium Tode

Several types of this organism have been isolated from the different soils. Vegetative hyphae usually colorless, creeping, branched; sclerotia spherical or elongated, singly or in masses, usually black, hard when dry, with a distinct cell division.

Sterile white mycelium

Snow-white sterile mycelium isolated repeatedly from all soils; growth floccose; hyphae hyaline, septate, branching. No conidial structures ever found; some types were found to form chlamydospores.

Sterile red mycelium

Hab. Isolated from garden, forest and Oregon soils.

Summary of Morphological Studies

Altogether there were isolated from the soil over one hundred distinct species of fungi, belonging to 31 genera. Some of the organisms have been grouped together, because the different types either did not show enough distinctive characters, or were not thought important enough to be separated into different species. There were several organisms left not included in the present description, due to the difficulty of their identification. The writer tried to avoid as much as possible the making of new species, leaving it to the special students on fungi.

The following genera of fungi were found to be represented in the soil:

1. Absidia
2. Mucor
3. Rhizopus
4. Zygorhynchus
5. Saccharomyces
6. Chaetomium
7. Monilia
8. Oidium
9. Sporotrichum
10. Botrytis
11. Aspergillus
12. Penicillium
13. Scopulariopsis
14. Verticillium
15. Cephalosporium
16. Trichoderma
17. Acrostalagmus
18. Zygodesmus
19. Dicoccum
20. Cephalothecium
21. Basisporium
22. Dematium
23. Acremoniella
24. Cladosporium
25. Alternaria
26. Fusarium
27. Melanconium
28. Coniothyrium
29. Sclerotium
30. Sterile white mycelium
31. Sterile red mycelium

The most common genera of fungi, as to numbers and species, found in the soils investigated are, in the order of their occurrence, as follows: Penicillium, Mucor, Aspergillus, Trichoderma, Cladosporium, Fusarium,

Cephalosporium, Rhizopus, Zygorhynchus, Acrostalagmus, Alternaria, and Verticillium. Going back to the work of other investigators on soil fungi, these genera are found, with one or two exceptions, also isolated by Jensen (53) and Dale (27). Hagem (43) mentions the Penicillia, Aspergilli and Mucors, as the three largest groups of soil fungi.

TABLE III

ISOLATION OF COMMON GENERA OF SOIL FUNGI BY DIFFERENT INVESTIGATORS

Genus	Koning	Dale	Jensen	Goddard	McLean and Wilson	Author
Acrostalagmus	*	..	*	*	..	*
Alternaria	*	*	*	..	*	*
Aspergillus	*	*	*	*	*	*
Cephalosporium	*	..	..	..	..	*
Cladosporium	..	*	*	*	*	*
Fusarium	..	*	..	*	..	*
Mucor	*	*	*	*	*	*
Penicillium	*	*	*	*	*	*
Rhizopus	..	*	*	*	*	*
Trichoderma	*	*	*	*	*	*
Verticillium	..	*	..	*	..	*
Zygorhynchus	..	*	*	..	*	*

As seen from the results as expressed in Table III, the genera Aspergillus, Mucor, Penicillium, and Trichoderma have been isolated by all six investigators of soil fungi, and hence represent a flora studied at different parts of the world. The other genera are reported by two or more investigators. These 12 genera are all reported by the author, as isolated from most soils studied; Koning (80) found 7 out of these 12, Dale (27) 10, Jensen (53) 9, Goddard (41) 9, and McLean and Wilson (73) 8. Altogether over 50 genera of fungi have been reported to have been isolated from the soil; most of then having been isolated in one, two, or more cases. Not enough work has yet been done on the subject to enable one to say with certainty that these are the soil genera; but as far as the work done shows, the Aspergilli, Mucors, Penicillia, and Trichodermae seem to be found in all soils.

As to particular species of soil fungi found in the different soils studied, it is not so easy to decide which species are actually soil forms and are found in all the soils; first, because the environmental conditions are so different that some forms found in one locality, under one particular set of conditions, may not be found in another; secondly, the different investigators might differ in their opinion as to the identification of a particular species, especially since some of them are very variable, and the methods of study, such as media, incubation, etc., are not standardized. For that reason, it is better to speak of groups of organisms as soil

forms, rather than as individual species. But some species are found to be reported by many investigators, as having been isolated from the soil at different parts of the world. For example, *Trichoderma Koningi*, *Cladosporium herbarum*, *Mucor racemosus*, and *Rhizopus nigricans* have been reported from different parts of the world. Several species of Mucors have been isolated by Hagem (42) in Norway, by Dale (27) in England, and by Jensen (53) and the author in this country.

The question then arises: "Is there any so-called fungus flora of the soil?" The data at hand do not as yet warrant any positive conclusion. But considering the fact that there are groups of soil fungi which were found in all, or at least, most soils studied, whether acid, neutral, or alkaline, whether cultivated or not, under different climatic conditions, one might conclude that if not all forms, or even most of them, there are some fungi which are always found in the soil, and these are soil fungi proper.

The fact that the genera Aspergillus, Mucor, Penicillium, and Trichoderma were found in all the investigations, where a complete survey of the soil flora was made, leads one to think that these organisms, associated with others, make up the fungus flora of the soil. A hypothetical soil flora should then consist of several species of Aspergilli, more than those in types (not always in numbers) of Mucors and Penicillia, together with the green Trichodermae; besides these, one would expect to find a Zygorhynchus, one or two species of Cladosporia, Alternaria, Rhizopus, Fusaria, Verticillia, Cephalosporia, associated perhaps with an Acrostalagmus, a Scopulariopsis, a Botrytis, some Sterile Mycelium, and one or two Yeasts. This would probably form the average fungus flora, to which of course should be added organisms found only in one particular soil and not found in another.

Of course, attention might be called to the fact that all the forms ascribed to one investigator have been isolated by him, not from one soil, but from several soil types; this fact is due to the taking of the samples only in one spot, at one time, and in one season of the year. Had the samples been taken repeatedly and from different parts of the field, the other forms also might have been isolated. It is the author's belief that the larger the number of samples taken from one soil for study, the nearer the fungus flora found would approach the average flora outlined above. Of course, the species should be studied, not the genera alone, because only the species found can give a true basis for comparison, but the stress should be laid, not only on the occurrence of the particular species, however important this might be, but also on the occurrence of the group of which the species is a representative. This will help us to get a clear insight into the flora of the soil.

PHYSIOLOGICAL STUDIES ON SOIL FUNGI

A. Nitrogen Fixation

Mannite solution was made up according to the following formula (70):

Distilled water	1000 c.c.
K_2HPO_4	0.20 gm.
$MgSO_4$	0.20 gm.
$CaCl_2$	0.02 gm.
$FeCl_3$	Trace
Mannite	15.00 gm.

This was distributed into 250-c.c. Erlenmeyer flasks, 100 c.c. of the solution in each flask. After the flasks were sterilized, they were inoculated with the desired organisms and allowed to incubate for 30 days at 25° C. The contents of the flasks were then transferred to Kjeldahl flasks and the regular Kjeldahl determinations made. The results are given in Table IV.

TABLE IV

NITROGEN FIXED PER 100 C.C. MANNITE SOLUTION

Name of Organism	Total mg. N.	Duplicate determination mg. N.	Average mg. N.	Minus check mg. N.
Penicillium luteum	.735	1.029	.882	0.280
Penicillium sp.	.735	.735	.735	0.133
Alternaria sp.	.588	.882	.735	0.133
Rhizopus nigricans	.882	.735	.809	0.207
Cephalothecium roseum	.588	.441	.515	—0.087
Check	.616	.588	.602	.....

The amount of nitrogen fixed lies within the analytical error and is negligible. The results confirm the investigations of Goddard (41) and others, who could not prove any nitrogen fixation by fungi.

B. Ammonification

For the determination of the ammonifying power of fungi the following method was used: 100 gm. of sieved air-dry soil of a light loamy texture were placed in 250-c.c. Erlenmeyer flasks; 155 mg. of nitrogen in the form of dried blood and cottonseed meal were added to the soil and well mixed in by shaking the flask thoroughly; 20 c.c. of water were then added to the flask, this amount forming about 70 per cent of the moisture-holding capacity of the soil. The flasks were then numbered, plugged with cotton, and sterilized in the autoclave at 15 pounds pressure for 15 minutes. The organisms used for inoculation were grown for 6 to 10 days on the No. II liquid medium, or Czapek's solution. The liquid culture of the organism was well shaken, and 1 c.c. of the spore-containing liquid was used for inoculation. The flasks were then incu-

bated at 22° C. for 12 days, since this period was found by the author (115) to be the most favorable one for the comparison of the ammonifying power of the different organisms. After that period the soil was transferred with enough water into copper flasks, and distilled over with MgO into standard HCl solution.

TABLE V

AMMONIA ACCUMULATION BY FUNGI IN 12 DAYS

Name of Organism	Dried Blood as source of N.				Cottonseed Meal as source of N.			
	Ammonia N. found. mg. N.		Average	Increase over check	Ammonia N. found. mg. N.		Average	Increase over check
Mucor racemosus	20.24	16.98	18.61	15.91	50.12	54.48	52.30	48.10
Mucor plumbeus	12.42	15.36	13.89	11.19	47.72	40.50	44.11	39.91
Mucor hiemalis I	20.42	16.80	18.61	15.91	38.97	36.17	37.57	33.37
Mucor hiemalis II	18.00	16.28	17.14	14.44	40.14	33.50	36.82	32.63
Rhizopus sp. I (B. 2)	23.35	21.15	22.25	19.55	47.11	51.47	49.29	45.09
Rhizopus sp. II	20.76	20.36	20.56	17.86	50.68	46.24	48.46	44.26
Rhizopus sp. (B. 5)	33.20	30.02	31.62	28.92	52.69	46.69	49.69	45.49
Rhizopus sp. (B. 3)	16.46	14.16	15.31	12.61	43.74	43.74	43.74	39.54
Rhizopus sp. (C. 5)	40.16	38.00	39.08	36.38	56.75	52.11	54.43	50.23
Zygorhynchus Vuilleminii	12.54	10.18	11.36	8.66	47.58	45.18	46.38	42.18
Aspergillus sp. (B. 16)	21.20	24.20	22.70	20.00	31.60	33.64	32.62	28.42
Penicillium luteum	4.59	4.99	4.79	2.09	14.40	14.14	14.27	10.07
Penicillium lilacinum	25.12	27.20	26.16	23.46	28.12	26.00	27.06	22.86
Penicillium chrysogenum	23.00	25.02	24.01	21.31	23.13	25.33	24.23	20.03
Penicillium sp. (9-24)	17.28	16.20	16.74	14.24	37.18	39.54	38.36	34.16
Penicillium decumbeus	3.89	3.59	3.69	0.99	16.71	17.11	16.91	12.71
Penicillium lividum	5.10	4.24	4.67	1.97	17.67	20.07	18.87	14.67
Penicillium sp. (D. 12)	15.41	15.41	15.41	12.71	24.13	26.52	25.32	21.12
Penicillium sp. (C. 7)	72.12	81.16	76.64	73.94	50.13	46.53	48.33	44.13
Penicillium Pfefferianus	3.72	3.96	3.84	1.14	10.92	9.24	10.08	5.88
Penicillium digitatum	6.00	7.20	6.60	3.90	31.60	30.20	30.90	26.70
Penicillium glaber	6.90	6.50	6.70	4.00	14.20	14.80	14.50	10.30
Penicillium italicum	8.10	8.30	8.20	5.50	25.40	27.00	26.20	22.00
Trichoderma Koningi	87.65	95.85	91.75	89.05	79.12	81.73	80.43	76.23
Monilia sitophila	40.12	42.24	41.18	38.48	58.21	61.60	59.91	55.71
Cephalothecium roseum	36.24	38.84	37.54	34.84	53.30	56.29	54.80	50.60
Cephalosporium sp. (B. 7)	39.15	39.75	39.45	36.75	50.12	52.72	51.42	47.22
Acrostalagmus cinnabarinus	40.20	44.20	42.20	39.50	72.60	74.72	73.66	69.46
Dematium pollulans	22.25	22.25	22.25	19.55	48.09	50.48	49.29	45.09
Alternaria humicola	53.04	61.13	57.09	54.39	64.02	68.46	66.24	62.04
Cladosporium herbarum	16.12	16.76	16.44	13.74	36.80	38.83	37.82	33.62
Fusarium caudatum	33.30	39.30	36.30	33.60	55.20	58.37	56.79	52.59
Bacterium mycoides	25.55	23.16	24.36	21.46	21.12	25.73	23.43	19.23
Bacillus proteus	10.00	10.10	10.05	7.35	11.52	13.71	12.62	8.42

Looking through Table V, one can readily see that all the fungi examined for their ability to decompose organic matter and liberate ammonia, do it quite readily. When the fungi are compared with the bacteria tested for their power to accumulate ammonia, many of them are found to be much stronger ammonifiers even than *Bacterium mycoides*, which is supposed to be a strong ammonifying organism. A great variation in

TABLE VI

INFLUENCE OF AGE OF CULTURE UPON THE AMMONIFYING POWER OF FUNGI

Name of Organism	Young Cultures								Cultures Six Months Older							
	Dried Blood				Cottonseed Meal				Dried Blood				Cottonseed Meal			
	Ammonia N. found		Average mg. N.	Increase over check mg. N.	Ammonia N. found		Average mg. N.	Increase over check mg. N.	Ammonia N. found		Average mg. N.	Increase over check mg. N.	Ammonia N. found		Average mg. N.	Increase over check mg. N.
	mg. N.	mg. N.			mg. N.	mg. N.			mg. N.	mg. N.			mg. N.	mg. N.		
Mucor racemosus	20.24	16.98	18.61	15.91	50.12	54.48	52.30	48.10	48.00	45.20	46.60	43.30	69.60	76.30	72.95	68.25
Mucor plumbeus	12.42	15.36	13.89	11.19	47.72	40.50	44.11	39.91	27.00	29.16	28.05	24.75	36.00	38.20	37.10	32.40
Mucor hiemalis	20.42	16.80	18.61	15.91	38.97	36.17	37.57	33.37	24.10	26.70	25.40	22.10	38.20	36.30	37.25	32.65
Rhizopus nodosus	40.16	38.00	39.08	36.38	56.75	52.11	54.43	50.23	15.60	13.50	14.55	11.25	42.60	37.50	40.05	35.35
Zygorhynchus Vuilleminii	12.54	10.18	11.36	8.66	47.58	45.18	46.38	42.18	22.50	28.30	25.40	22.10	66.10	61.70	63.90	59.20
Penicillium chrysogenum	23.00	25.02	24.01	21.31	23.13	25.33	24.23	20.03	11.20	7.60	9.40	6.10	27.00	26.20	26.60	21.95
Penicillium luteum	4.59	4.99	4.79	2.09	14.40	14.14	14.27	10.07	4.80	4.20	4.50	1.70	3.20	3.60	3.40	—1.00
Penicillium lilacinum	25.12	27.20	26.16	23.46	28.12	26.00	27.06	22.86	7.50	7.70	7.60	4.80	9.00	10.40	9.70	5.30
Penicillium sp. (9-24)	17.28	16.20	16.74	14.24	37.18	39.54	38.36	34.16	7.80	6.20	7.00	4.20	10.40	9.20	9.80	5.40
Penicillium lividum	5.10	4.24	4.67	1.97	17.67	20.07	18.87	14.67	2.10	1.90	2.00	—0.80	10.60	11.20	10.90	6.50
Penicillium sp. (C. 7)	72.12	81.16	76.64	73.94	50.13	46.53	48.33	44.13	24.70	21.90	23.30	20.00	29.80	30.60	30.20	25.50
Cephalothecium rozeum	36 24	38.84	37.54	34.84	53.30	56.29	54.80	50.60	30.40	25.20	27.80	24.50	51.20	54.40	52.80	48.10
Acrostalagmus cinnabarinus	40.20	44.20	42.20	39.50	72.60	74.72	73.66	69.46	16.50	13.70	15.10	11.80	60.60	58.20	59.40	54.70
Dematium pollulans	22.25	22.25	22.25	19.55	48.09	50.48	49.29	45.09	10.60	12.02	11.31	8.01	38.12	42.43	40.28	35.58

the ammonifying power is found between the different groups of organisms, the strongest one being *Trichoderma Koningi*, a very common soil form, liberating in 12 days, in the form of ammonia, 57.5 per cent of the nitrogen in the dried blood, and 49.2 per cent in the cottonseed meal. The Penicillia proved to be, as a rule, weak ammonifiers, with only one exception. The great variation between the different Penicillia in the ammonifying power is probably due to the fact that the genus is so large, and that there are combined many organisms only distantly related. For that reason such a variation could be found as between the *Penicillium decumbens* liberating, in the form of ammonia, 0.6 per cent of the nitrogen in dried blood and 8.2 per cent of the nitrogen in cottonseed meal, and *Penicillium* sp. (C. 7) liberating, in the form of ammonia, 47.7 per cent of the nitrogen in dried blood and 28.5 per cent of the nitrogen in cottonseed meal, under the same environmental conditions and in the same period of time.

The ammonifying power of the Mucorales, including representatives of the genera Mucor, Rhizopus, and Zygorhynchus, show less variation than the Penicillia. The extremes of these groups represent *Rhizopus* sp. (C. 5), with the accumulation of nitrogen, in the form of ammonia, 23.5 per cent in the dried blood and 32.4 per cent in the cottonseed meal, and *Zygorynchus Vuilleminii* with 5.6 per cent in the dried blood, and *Mucor hiemalis* with 21 per cent in the cottonseed meal. All the other fungi tested gave fairly large accumulation of ammonia, including the Monilia, Alternaria, Fusaria, Cephalosporia, and the others.

Some of the organisms, having been kept in culture on artificial media for six months, were tested again for their ammonifying power. The results are given in Table VI.

It is seen from this table that the different groups of organisms behaved differently when kept in culture on artificial media for a certain length of time. The Mucorales decreased their power to ammonify only in very few instances, while in several cases a stronger power to liberate ammonia is found after the organisms have been kept in culture for six months, than soon after they have been isolated from the soil. The reverse is true with the Penicillia, some of which seemed to have lost almost entirely their ammonifying power, while, with only one exception, every one of them gave, with both dried blood and cottonseed meal, smaller quantities of ammonia at the later period. The few other organisms, besides the Mucorales and the Penicillia, which have been tested, behaved like the Penicillia, e. g., decreased in their power to liberate free ammonia after being kept in culture on artificial media for six months. However, the difference in numbers of spores used for inoculation might have had something to do with the difference in results.

C. Diastase Secretion and Cellulose Decomposition

As was seen from the brief historical review presented in the first part of the paper, the fungi seem to play an important part in the decomposition of cellulose. Out of the fungi isolated from the soil the author selected 22 species, representing the different groups of the soil fungi, to be tested out for their power to hydrolize starch and decompose cellulose. Starch and cellulose agar were made up according to the formulae given by McBeth and Scales (72). The organisms were inoculated into sterile Petri dishes containing the proper agar; the starch plates were then incubated for 6 days at 22° C., and the cellulose plates for 15 days at 30° C. The starch plates were examined after 6 days for the production of an enzymic ring around the colony. This was clearly seen even with the naked eye, but a few drops of iodine solution well defined the enzymic zone. The cellulose plates were examined under the microscope, and where cellulose decomposition had taken place the threads could be found broken down and destroyed.

TABLE VII

DIASTASE SECRETION AND CELLULOSE DECOMPOSITION BY SOIL FUNGI[1]

Name of Organism	Enzymic zone of diastase activities in 6 days	Cellulose decomposition in 15 days
Penicillium sp. (9-24)	—	++
Penicillium sp. (B. 9)	—	++
Penicillium sp. (B. 12)	—	++
Penicillium decumbens	—	+
Penicillium digitatum	6 mm.	—
Scopulariopsis brevicaule, var.	4 mm.	+
Penicillium glaber	1.5 mm.	+
Aspergillus fumigatus	—	++
Aspergillus diversicolor	—	—
Aspergillus sp. (B. 16)	2 mm.	+
Aspergillus calypiratus	1 mm.	++
Absidia sp.	—	—
Mucor plumbeus	—	—
Mucor hiemalis	—	—
Rhizopus nodosus	—	—
Melanconium sp.	—	++
Trichoderma Koningi	—	++
Verticillium sp.	6 mm	++
Basisporium gallarum	—	++
Alternaria humicola	—	++
Fusarium bullatum	—	++
Cephalosporium curtipes (?)	—	—

[1] + indicates cellulose decomposition. ++ very strong decomposition. — no cellulose decomposition, also no diastase secretion.

Comparing the data presented in Table VII, one can see that the organisms do not have necessarily combined the power to secrete diastase and decompose cellulose. Some of the strongest cellulose decomposing organisms, such as the Melanconium, Trichoderma, Fusarium, and

others, did not secrete any diastase under the conditions at hand, and no clearing was produced in the starch around the colony. The four Mucorales tested neither hydrolized the starch nor decomposed the cellulose.

Summary of the Physiological Studies

1. Five fungi isolated from the soil and representing distinct groups of organisms were not found to fix any appreciable quantities of atmospheric nitrogen, which would not lie within the analytical error.

2. The fungi of the soil are very strong ammonifiers, most of them liberating larger quantities of ammonia than the strong ammonifying bacteria, when tested under similar conditions.

3. The *Trichoderma Koningi* proved to be, under the conditions at hand, the strongest ammonifying organism; the Penicillia differing with the different species, most of them being comparatively weak ammonifiers; the Mucorales are fairly strong ammonifiers, the different species not differing so much from one another as the Penicillia.

4. The growing of the organisms on artificial culture media for six months affected the ammonifying power of the organisms differently: while that of the Mucorales was hardly affected, or was even beneficial, that of the Penicillia and other organisms tested, was detrimental, their power decreasing with almost all organisms.

5. Most of the fungi are very strong cellulose decomposers, 15 out of 22 organisms tested prove to decompose the cellulose rather rapidly; most of the fungi have a rather weak ability to secrete diastase, only 6 out of 22 organisms forming an enzymic ring in the starch medium.

GENERAL SUMMARY

1. The fungi of the soil represent a numerous group of organisms found in all the soils studied in numbers large enough to warrant a conclusion that they probably play an important part in the fertility of the soil.

2. There does not seem to be any distinct difference between the species of fungi found in cultivated soils and those in uncultivated soils, though each soil seems to have a more or less characteristic fungus flora: for example, the cultivated orchard soil has a great abundance of Mucorales, while the forest, uncultivated soil, has an abundance of Penicillia and Trichodermae. This might be due rather to the soil reaction, methods of manuring, and crop grown upon the soil, than to the cultivation itself.

3. The numbers of fungi decrease rapidly with depth, so that at 12 to 20 inches below the surface very few fungi can be found, the largest numbers occurring within the upper four inches of soil. As to the species, no distinct differences among the organisms were found with the different soil depths, except that in the subsoils of most of the soils studied, *Zygorhynchus Vuilleminii* was found to be present often as the only organism, when soil was inoculated directly upon sterile medium.

4. Over one hundred distinct species of fungi were isolated from the soil, belonging to 31 genera, many of the species being isolated from several of the different soils.

5. Many pathogenic fungi, such as different Fusaria, Alternaria, Aspergilli, Coniothyrium, and others, have been isolated from the soil, a fact which leads one to think that they pass certain stages of their life history in the soil, or are able to live saprophytically in the soil, and perhaps play a part in its fertility.

6. The study of the physiological activities of the fungi pointed out the fact that they do not play a very great, if any, part in the fixation of atmospheric nitrogen, but they do prove to be able to decompose organic matter rapidly and liberate ammonia, under laboratory conditions. Many of them prove to be strong decomposers of cellulose, though fewer of them hydrolize starch.

The question, "Is there any so-called fungus flora of the soil?" cannot as yet be answered in the affirmative till more work has been done with soils collected from different parts of the world. But it is seen from the data at hand that there is a rather distinct fungus flora of the soils studied, and this holds particularly true with regard to certain organisms. The importance of the fungi in the soil seems to lie in the formation of humus and in the liberation of ammonia, which can then be utilized by the higher plants, either directly, or after it was changed by other organisms into nitrates. The numerous species of soil fungi isolated and the large numbers of them supply an impetus to a further study of these organisms, which will help to solve the problem of their importance in the soil.

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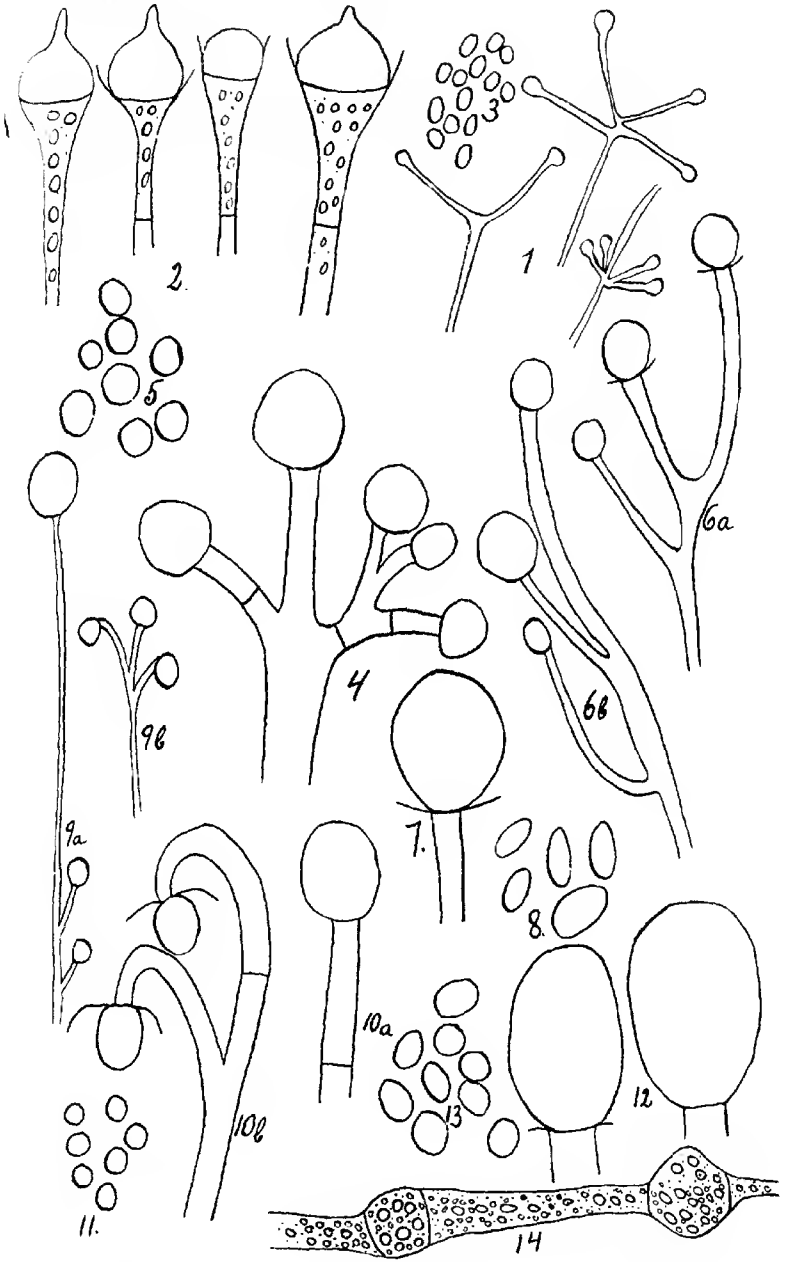
PLATE I

Fig. 1.—*Absidia Orchidis.* Different types of branching of the sporangiophore (x 104).
Fig. 2.—*Absidia Orchidis.* Types of columellae (x 600).
Fig. 3.—*Absidia Orchidis.* Spores (x 1200).
Fig. 4.—*Mucor botryoides.* Branching of sporangiophore, carrying columellae (x 600).
Fig. 5.—*Mucor botryoides.* Spores (x 1200).
Fig. 6 a-b.—*Mucor saturninus.* Sporangiophores (x 200).
Fig. 7.—*Mucor saturninus.* Columella (x 300).
Fig. 8.—*Mucor saturninus.* Spore (x 1200).
Fig. 9 a-b.—*Mucor* sp. (C. 44). Sporangiophores straight and curved type (x 104).
Fig. 10.—*Mucor* sp. (C. 44). Columellae of the straight and curved type of sporangiophores (x 1200).
Fig. 11.—*Mucor* sp. (C. 44). Spores (x 1200).
Fig. 12.—*Mucor* sp. (D. 28). Columellae (x 600).
Fig. 13.—*Mucor* sp. (D. 28). Spores (x 1200).
Fig. 14.—*Mucor* sp. (D. 28). Portion of mycelium, showing oil globules and chlamydospores (x 600).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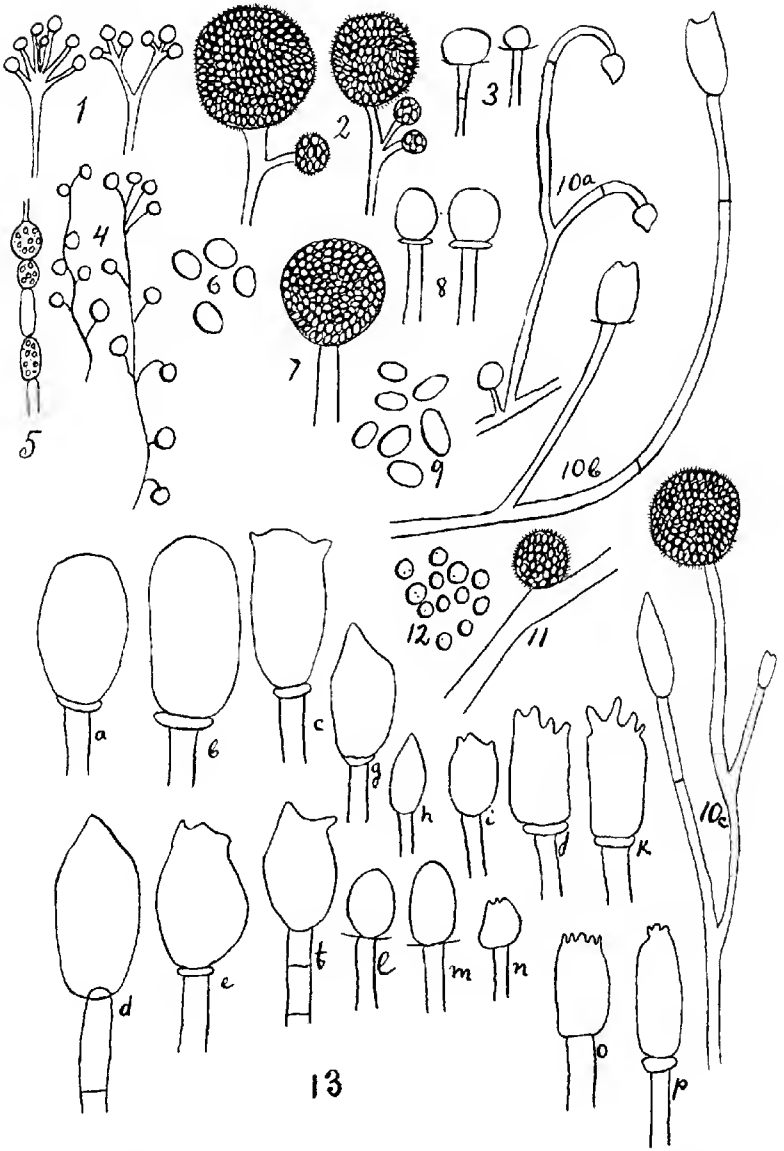
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PLATE II

Fig. 1.—*Mucor Glomerula.* Branching of Sporangiophores (x 60).
Fig. 2.—*Mucor Glomerulo.* Sporangia (x 310).
Fig. 3.—*Mucor Glomerula.* Columellae (x 310).
Fig. 4.—*Mucor circinelloides.* Sporangiophores branched sympodially (x 60).
Fig. 5.—*Mucor circinelloides.* Portion of mycelium, showing chlamydosphores (x 310).
Fig. 6.—*Mucor circinelloides.* Spores (x 1200).
Fig. 7.—*Mucor hiemalis.* Sporangium (x 310).
Fig. 8.—*Mucor hiemalis.* Columellae (x 310).
Fig. 9.—*Mucor hiemolis.* Spores (x 1200).
Fig. 10.—*Mucor plumbeus,* showing branching of sporangiophores, 10 c, showing one sporangium and two columellae (x 310).
Fig. 11.—*Mucor plumbeus.* Portions of sporangiophore bearing a sessile sporangium (x 310).
Fig. 12.—*Mucor plumbeus.* Spores (x 600).
Fig. 13 a-p.—*Mucor plumbeus.* Types of columellae (x 600). a, o, p from Strain I; b, c, g, h from Strain IV; e, f, d from Strain (C. 20); l, m, n from Strain (D. 23); j, k from Strain (C. 22).

PLATE III

Fig. 1 a, b, c.—*Zygorhynchus Vuilleminii.* Formation of zygospores (x 310).

Fig. 2.—*Zygorhynchus Vuilleminii.* Portion of sporangiophore, showing ripe sporangium and zygospore (x 600).

Fig. 3.—*Zygorhynchus Vuilleminii.* Branching of sporangiophore (x 600).

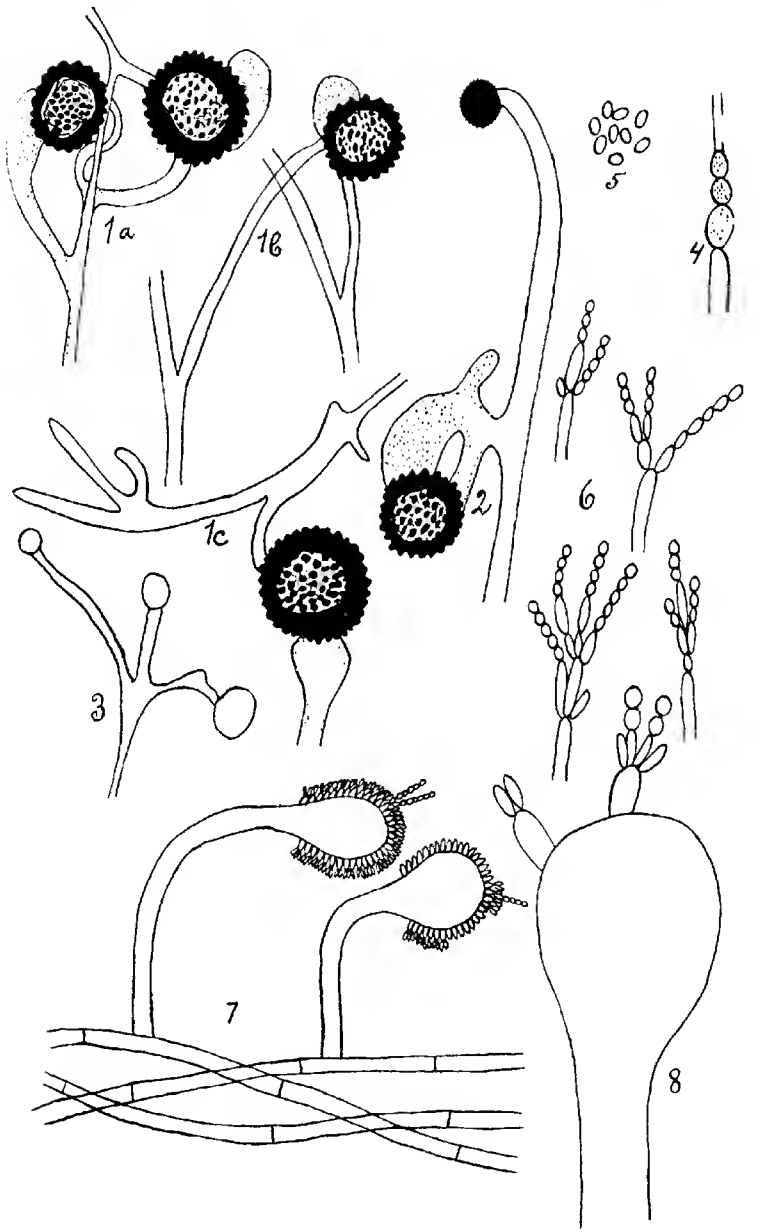
Fig. 4.—*Zygorhynchus Vuilleminii.* Portion of mycelium showing chlamydospores (x 310).

Fig. 5.—*Zygorhynchus Vuilleminii.* Spores (x 1200).

Fig. 6.—*Monilia humicola.* Portions of conidiiferous branches showing formation of conidia (x 600).

Fig. 7.—*Aspergillus* sp. (C. 19). Vegetative hyphae and conidiophores (x 310).

Fig. 8.—*Aspergillus* sp. (C. 19). Greatly enlarged swelling of conidiophore showing sterigmata and conidia (x 1200).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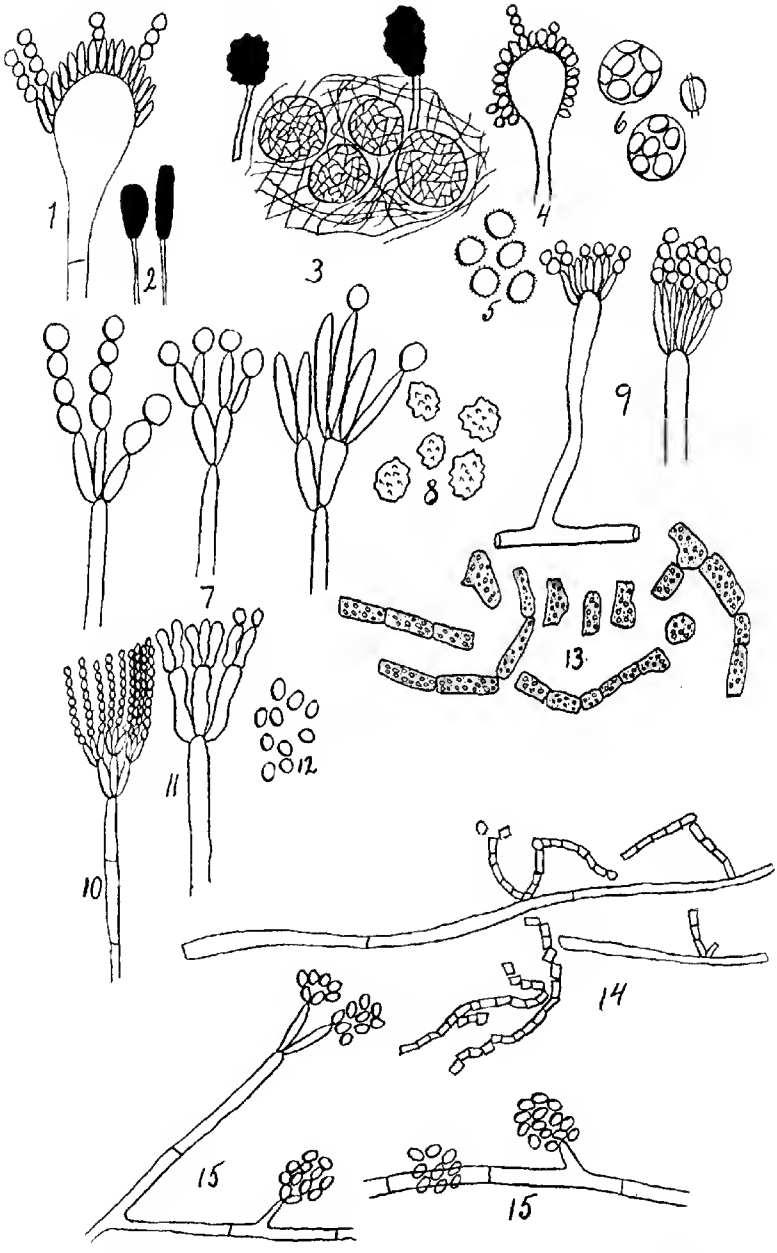
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PLATE IV

Fig. 1.—*Aspergillus calyptratus.* Portion of conidiophore showing swelling, sterigmata, and conidia (x 1200).

Fig. 2.—*Aspergillus calyptratus.* Conidial fructifications (x 104).

Fig. 3.—*Aspergillus repens* (from raisin agar). A portion of the mycelial surface with perithecia and canidiophores (x 104).

Fig. 4.—*Aspergillus repens.* Conidial swelling with sterigmata (x 600).

Fig. 5.—*Aspergillus repens.* Conidia (x 1200).

Fig. 6.—*Aspergillus repens.* Asci containing the ascospores also free ascospore (x 1200).

Fig. 7.—*Scopulariopsis brevicaule* (n. var. ?) Conidiophores with conidial chains of smooth spores (x 600).

Fig. 8.—*Scopulariopsis brevicaule* (n. var. ?) Echinulate spores (x 600).

Fig. 9.—*Penicillium glaber.* Portions of conidiophores showing sterigmata and spores (x 600).

Fig. 10.—*Penicillium desiscens.* Portion of conidiophore magnified (x 600).

Fig. 11.—*Penicillium desiscens.* Highly magnified portion of conidiophore to show sterigmata (x 1200).

Fig. 12.—*Penicillium desiscens.* Spores (x 1200).

Fig. 13.—*Oidium* sp. (A. 30). The breaking of the hyphae into oidia (x 570).

Fig. 14.—*Oidium lactis.* Hyphae and abundant formation of side branches, resulting in the formation of odia (x 310).

Fig. 15.—*Cephalosporium* sp. (D. 32). Formation of conidial heads at the tip of the branches or on the main hyphae (x 600).

PLATE V

Fig. 1.—*Cephalosporium* sp. (G. 23). Branching of conidiophore and formation of heads of conidia at the tip of the branches (x 310).

Fig. 2.—*Cephalosporium* sp. (G. 23). Spores (x 1200).

Fig. 3.—*Cephalosporium* (n. sp. ?) (C. 56). Branching of sporangiophore and the grouping of spores into heads (x 600).

Fig. 4.—*Cephalosporium* (n. sp. ?) (C. 56). Branching of conidiophore, showing heads of spores surrounded by slime in heads (x 600).

Fig. 5.—*Cephalosporium* (n. sp. ?) (C. 56). Spores (x 1200).

Fig. 6.—*Zygodesmus* sp. Portion of mycelium with side branches carrying spores (x 104).

Fig. 7.—*Zygodesmus* sp. Tips of the branches of the conidiophore, surrounded by spores (x 600).

Fig. 8.—*Zygodesmus* sp. Three spores of the major strain Oc (x 1200).

Fig. 9.—*Zygodesmus* sp. Four spores of the minor strain B (x 1200).

Fig. 10.—*Coniothyrium Fuckelii* (?). Pycnidium surrounded by mycelium (x 60).

Fig. 11.—*Coniothyrium Fuckii.* (?) Portion of mycelium (x 310).

Fig. 12.—*Coniothyrium Fuckelii* (?) Spores (x 600).

Fig. 13.—*Cladosporium epiphyllum.* Portion of fruiting hyyhae and fructifications.


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SOURCES OF ERROR IN SOIL BACTERIOLOGICAL ANALYSIS[1]

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Investigations in the field of soil biology have concerned themselves mostly with the measurement of the activity of microörganisms by means of chemical methods. To ascertain to what degree the various bacteriological phenomena of ammonification, azofication and azotofication,[2] etc., have taken place, recourse has been taken to the usual qualitative and quantitative methods of the analytical chemist.

One of the first surprises in store for the soil biologist who, as a rule, has had more or less training in analytical chemistry, is to the effect that good duplicate determinations, in the chemical sense, are the exception rather than the rule.

No action which depends upon vital processes for its performance can be repeated with the certainty of chemical reaction, since the slightest differences in environmental factors cause variation in the response of the micro-flora and fauna involved. Small irregularities are magnified rather than reduced when the bacteriological activity is eventually measured by chemical methods. Greater or less differences between duplicates may result from wholly obscured causes, so that the problem of obtaining closely agreeing determinations in biological work resolves itself, in the main, into one of eliminating errors in methods of technique.

Probably all investigators have taken care to use soil containers of the same type throughout one comparative investigation. In order to determine to what extent differences in the size and shape of the soil container would make manifest differences in the results, the following experiment was carried out.

Ammonification tests were made with 100-gm. portions of Norfolk sandy loam soil to which had been added 5 gm. of dried blood. The soil portions were made up to optimum moisture content, 13 per cent, with the addition of 3 c.c. of water for each gram of organic matter. Dupli-

[1] Received for publication April 15, 1916.

[2] Lipman, J. G. Suggestions concerning the terminology of soil bacteria. *In* Bot. Gaz., v. 51, p. 454-460, 1911.

cate charges of the soil thus prepared were placed in seven different types of containers, incubated for 7 days at 22° C, and the ammonia accumulated was determined by the magnesium oxide distillation method.

The influence of the size and shape of the vessel on ammonification, all other factors being alike, is shown in Table I.

TABLE I

AMMONIFICATION IN CONTAINERS OF DIFFERENT TYPES

Lab. No.	Type of Container	Ammonia accumulated		
		Mg. N.	Mg. N.	Average
1-2	200 c.c. Erlenmeyer.....	67.82	68.04	67.93
3-4	250 c.c. Beaker	53.54	54.61	54.07
5-6	300 c.c. Erlenmeyer.....	74.02	87.70	80.86
7-8	500 c.c. Erlenmeyer.....	116.30	119.14	117.72
9-10	250 c.c. Florence.....	76.70	76.70	76.80
11-12	250 c.c. Salt mouth bottle.....	63.76	63.03	63.38
13-14	275 c.c. Tumbler.....	34.87	71.70	78.29

Apparently there exists an intimate correlation between the aeration of the soil and its ammonifying power. A difference of over 100 per cent exists between the ammonia accumulated in a 250 c.c. beaker and a 500 c.c. Erlenmeyer flask. Even between beakers, bottles, tumblers, all of approximately 250 c.c. capacity, there exists differences in some cases as great as 46 per cent. Of considerable interest is the fact that the soil portion in the 250-c.c. bottles had an ammonifying power greater than the same soil in a 250-c.c. beaker. The soil depth in the former was approximately two and one-half times as great as that in the beakers, nevertheless, more ammonia accumulated at the end of the incubation period. As can be seen from the table there is a gradual increase in the ammonifying power of the soil as the size of the Erlenmeyer flasks was increased from 200 to 500 c.c. The soil in the tumbler has a higher ammonifying power than that in any of the other containers, with the exception of the 500-c.c. Erlenmeyer flask.

Considering the ease with which the soil can be removed from this utensil, and the generally higher results obtained, together with its ability to withstand several usages, it would seem that this should be adopted as a vehicle for all biological work other than that of a pure culture nature.

The necessity, then of using containers of the same size and shape in all comparative studies becomes at once apparent. Likewise in comparing the results of various workers and the relative percentages of ammonia accumulated, it is not impossible that erroneous deductions may be made, due to no more important a factor than the size and shape of the containing vessel.

METHOD OF MIXING SOIL AND ORGANIC MATTER

Most laboratory guides recommend stirring with the soil, by means of a spatula, all materials designated to bring out specific biological activities, in order to effect a uniform mixture of the same. Where the fresh soil method[1] is used for the determination of biological activities one encounters considerable difficulty in obtaining an even incorporation of the materials to be used in the carrying out of the experiment. Wide deviations between duplicate determinations are not uncommon in such work, and to overcome these difficulties the writers have made use of the metal shakers shown in figure 1.

This shaker is commonly used in the mixing of beverages for which purpose it is extensively advertised. The metal prongs fixed to the cover serve to stir up the larger soil aggregates, thus effecting a very intimate and uniform incorporation of the specific materials with the soil. Very satisfactory results have been obtained by the use of a tumbler and the cup of the shaker with a pinch-cock inserted to churn the soil mass (fig. 1).

In order to demonstrate the advantages of the soil shaker in obtaining close duplicate determinations, the following comparison of the shaker and spatula methods of incorporating organic matter into the soil has been made.

Two soils, a Norfolk sandy loam and a Penn clay loam were used. One-hundred-gram portions of soil were mixed with 3 gm. of dried blood by means of the two devices. Each soil was used both in its air-dry state and at its optimum moisture content. Two periods of mixing for 15 seconds and 30 seconds were used with both soils in the dry and in the moist conditions. Five parallels were run for each period of mixing in both the wet and dry soils. The data for each parallel, as well as the experimental error of the average of the five, are shown in Table II. While it is admitted that the number of instances is hardly large enough in each case to render the use of any statistical method legitimate, nevertheless, it serves as a good index to the authenticity of the average and serves to emphasize the point under discussion. These comparisons were made according to the formula that the probable error (p.e.) of the average

$$= \pm .6745 \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

[1] Loc. cit. p. 1.

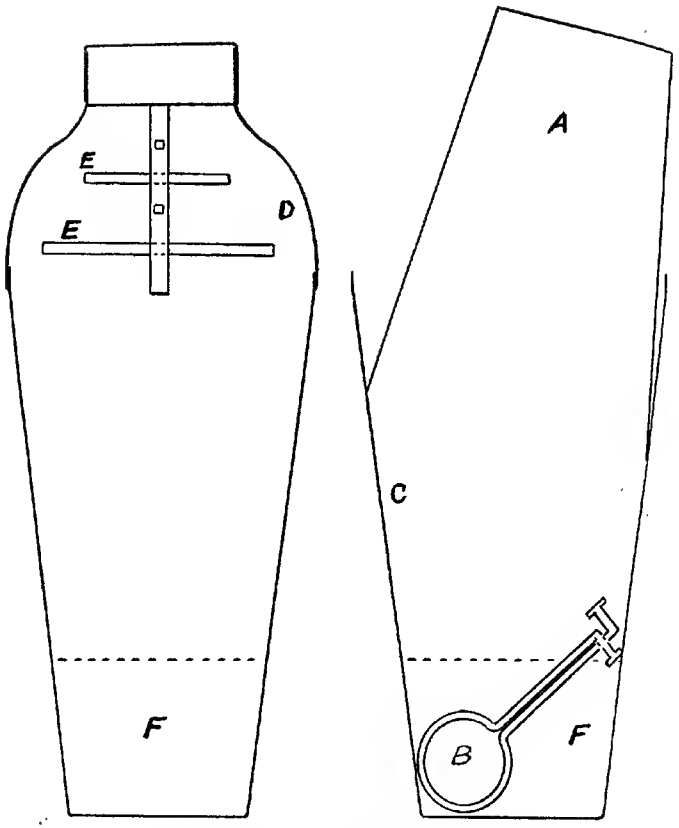

Fig. 1. Diagram of apparatus used for mixing soil.
A—Tumbler; B—Pinch-cock; C—Cup of the Shaker; D—Cap of the Shaker; E—Metal prongs for stirring the soil; F—Soil.

TABLE II

AMMONIFICATION TESTS OF DIFFERENT METHODS OF MIXING SOILS

Condition of soil at time of Mixing	Means of Mixing	Time of Mixing	Mg. N. accumulated as NH_3 in 7 days					
			Norfolk Sandy Loam			Penn Clay Loam		
			Det.	Average	Probable error of Av. + or —	Det.	Average	Probable error of Av. + or —
Air Dry	Shaker	30 sec.....	88.30			119.80		
			85.30			117.30		
			84.60			119.80		
			84.00			119.30		
			84.40	82.35	.521	120.80	119.40	.389
Air Dry	Shaker	15 sec.....	83.05			109.53		
			85.30			116.07		
			88.10			119.10		
			87.70			112.05		
			85.20	85.87	.622	121.69	115.68	1.452
Air Dry	Spatula	30 sec.....	87.60			113.20		
			73.10			118.20		
			73.90			123.00		
			75.10			121.80		
			74.30	76.80	1.835	120.00	119.24	1.163
Air Dry	Spatula	15 sec.....	70.90			116.90		
			75.82			115.10		
			83.83			113.80		
			72.57			104.80		
			78.50	76.32	1.542	117.00	113.72	1.516
Moist Soil	Shaker	30 sec.....	94.00			124.30		
			94.80			119.20		
			94.90			118.20		
			95.60			126.50		
			91.90	94.24	.428	124.30	122.50	1.085
Moist Soil	Shaker	15 sec.....	93.75			124.20		
			92.10			121.00		
			93.20			124.80		
			91.70			116.70		
			92.60	92.67	.248	122.70	121.88	.978
Moist Soil	Spatula	30 sec.....	93.90			116.00		
			94.40			122.00		
			100.40			120.15		
			97.90			113.50		
			96.60	96.64	.804	117.80	117.89	1.010
Moist Soil	Spatula	15 sec.....	76.70			127.30		
			65.60			118.50		
			76.50			122.10		
			74.80			119.60		
			84.00	75.52	1.975	120.90	121.68	1.032

While the data in the foregoing table are not entirely free from slight discrepancies, the following features are apparent.

1. Mixing the organic matter with the soil for 30 seconds shows a larger amount of ammonia accumulated than where the mixing occupied only 15 seconds.

2. The experimental error of the average for 30-second mixings is smaller than that obtained for 15-second mixing periods.

3. In general, there is not as great a difference between the two periods of mixing with the shaker as is the case where the spatula is used.

4. The averages obtained with the shaker are larger than where the spatula was used to stir the organic matter into the soil.

5. A comparison of the moist and the air-dry soil shows a larger accumulation of ammonia in the case of the former soil. Since the moist soil was prepared by adding water to the dry soil a few minutes before mixing, there should exist no legitimate differences in the soil flora to account for this difference. The more plausible explanation would be a better oxygen pressure in moist soil. The moist soils were uniformly compacted by placing a 500-gm. weight on the surface of the moist soil in the tumbler, after the organic matter had been admixed.

6. In general, the experimental error of the averages of the soils prepared in the shaker is smaller than that obtained with the spatula, which bespeaks a narrower variation in the duplicate determinations.

This device is also exceptionally well adapted to the incorporation of very small quantities of materials of a finely pulverized condition of the soil. Although not tabulated here, additional data concerning the incorporation of lime, fertilization, et cetera, has been obtained, indicating the superiority of this method over the spatula method so widely recommended.

The shaker is easily sterilized and by rinsing it out with HCHO or C_2H_5OH it can be used for carrying out research which precludes contamination.

Many other sources of error in bacteriological work are under investigation, and it is hoped cognizance may be taken of this preliminary data, which have proven of value both to ourselves and other workers in this laboratory, and that it may act as a stimulus for an improved bacteriological technique in further biological work.

STUDIES ON THE ACTIVITY OF SOIL PROTOZOA[1]

By

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The possibility of soil protozoa acting as a destructive agent in soil fertility still seems to be an unsettled question.

In the most recent publication by Goodey (4) in which he employed old soils that were stored for some years, some of which showed the absence of protozoa, developed in hay infusion, he concluded as follows: "The results of the experiments described above, lead me to the conclusion, that the protozoa, including cilicates, amebae, and flagellates added to the soil have not been able to act as a factor limiting bacterial activity in the soil. Inferentially, therefore, the ciliates, amebae and flagellates obtainable from ordinary soil under cultural conditions do not function as the limiting factor. This is in accord with and extends the conclusion put forward in my earlier paper (3), that the ciliated protozoa are present in soil in an encysted condition and cannot function, therefore, as the factor limiting bacterial activity."

Russell (12) in his criticisms of Goodey's researches is still of the opinion that the results of his previous work (14, 15) point to a factor which limits bacterial activities. He states, "The soil is known to be inhabited by numbers of bacteria, eel worms, vermes and numerous other organisms of higher orders and visible dimensions, and these lead active lives. Recently it has been shown in the Rothamsted Laboratory that a protozoan fauna also exists, some members at least of which are leading a trophic life." He (Russell) objects to the technique employed by Goodey in that, "The organisms inoculated into the soil are in the main those which figure largely in cultures made by adding soil to hay infusions. It has already been shown, however, (*v. supra*) that the culture fauna is distinct from the trophic soil fauna. There is, therefore, no evidence that the normal soil fauna was put back into the soil partially sterilized. On the contrary it apparently was not, nor is there evidence, except perhaps in one case, that the added organisms survived at all." * * * * "The protozoa are not obtained in pure culture alone, but they are added along with hay infusion and bacteria. Until more is known of the kind of protozoa occurring in the trophic state in the soil and their life history in the soil, it will be impossible to lay much stress on the negative results of re-infections."

[1] Received for publication May 31, 1916.

In a recent work, on soil protozoa, Waksman (19) summarizes in part as follows: "Soil protozoa do not have any appreciable influence upon the ammonification by bacteria. The presence of protozoa acts detrimentally upon bacterial numbers so that when the conditions become favorable for protozoa development, the baeterial numbers decrease."

With the exception of examining and finding active protozoa present after the incubation period, this work of Waksman's is subject to the criticisms offered by Russell of Goodey's research. In no case were protozoa free from bacteria and decomposition products of organisms in the soil extract used as the inoculum into the sterile soil when the destructive effect of the protozoa was to be noted. Sherman (17, 18) in his researches with soil protozoa also realized this criticism to his work. Moreover, it would hardly seem scientifically accurate to compare the ammonifying power of organisms of the soil (bacteria and actinomyces in the main) from a depth of 20 inches which were originally entirely under anaerobic conditions, with organisms from the surface soil, as was undertaken by Waksman. It would be expected that the aerobie organisms contained in the upper layer of soil would be much more vigorous.

It is generally known that the ammonifying power of a soil, varies as the percentage of moisture which it contains. The production of ammonia by a soil flora usually increases until the moisture content equivalent to the physical optimum is reached. With heavier soils, the maximum is slightly higher than the physical optimum. Soon after the optimum condition of moisture, where there is maximum ammonification, has been reached, there is an abrupt decrease in ammonifying power. This is explained by the fact that the conditions become unfavorable for decomposition, and thus the processes proceed very slowly under these conditions which approach the anaerobic state. A parallel instance to this might be seen in the above cited work. There is reason to believe that the bacteria which were introduced with the protozoa in the protozoa culture were better ammonifiers than the bacteria free from protozoa, hence the protozoa might have been the cause of considerable destruction of bacteria and still an increase in ammonia would be found, being probably due to bacteria alone, as is recorded with soil B with 14 and 28 per cent moisture (protozoa plus bacteria) (19, p. 144).

In a previous publication the writer (7) gives an account of studies in the length of time of excystation of soil protozoa and some of the factors which influence the presence of protozoa in the active state in the soil. He also examined field and greenhouse soils to get some idea as to the extent of the activity of the protozoa present. For several reasons which need not be mentioned here, the effect of the presence of organic matter and the physical property of the soil upon the presence of protozoa in soils, in the active state, could not be worked out and included in the last publication, consequently this paper was prepared for this reason in part.

Protozoan Activity in the Soil

The several methods used in studying protozoan activity in the soil were previously reviewed, hence no further mention will be made of them at this time.

Inasmuch as there were no extended data available which indicated the approximate time required for protozoa to excyst when the cysts come in contact with free water, the previous work (7) was carried out by making several examinations of the soils in question, for a period of two minutes only, thus eliminating the possible errors which might arise from the fact that the protective cysts might burst open during the examination and hence a false impression as to the real number of active protozoa in the soil would be obtained, as was suggested by Martin and Lewin (10). The writer realized that two minutes was a short period of time for a single examination, but when the field and greenhouse soils were examined for active protozoa for the actual length of time that the soil was under observation and in consideration of the number of samples taken, which was 14 or 15, it would seem that the active protozoa could have been detected and no doubt were, as will be demonstrated later. After making several thousand determinations, some of which were recorded in the last publication, to which reference has been made, and not finding any protozoa to excyst in 2 minutes, the 5 to 7 minute examination period was tried out and found to be satisfactory, as stated before (7, p. 486).

In the problems presented in this work, the direct examination of a small portion of soil was made for 5 minutes. Each soil was subjected to an examination extending from 15 to 20 minutes, i. e. 3 or 4 samples of each soil were taken. The low and the high power (4 mm.) of the microscope were employed. It was found that very great care must be exercised in employing the high power in determining active protozoa. There is still a chance for the investigator to err by employing the high power especially if there are protozoa which are as small as bacteria and have the Brownian movement, for in such a case it would be impossible to distinguish between protozoa and bacteria by this method. By extended examinations, it was found, however, with the soils employed, that practically all of the active protozoa could be seen with the low power.

In order to collect more data with regard to the influence of moisture content, physical character of the soil and the presence of organic matter upon the activity of protozoa in the active condition in the soil, 100-gm. portions of a typical Sassafras sandy loam soil which had been air dried, were weighed out into tumblers. The water-holding capacity of this soil was determined by the funnel method, which consists of merely determining the amount of water which is held by a weighed quantity of soil. It was found that 40 per cent of the amount of water held in this way by the soil, very closely approximated the physical optimum of the soil, deter-

TABLE I

PRESENCE OF ACTIVE PROTOZOA IN A SASSAFRAS SOIL, WHICH HAD RECEIVED TREATMENTS OF DRIED BLOOD AND COW MANURE, AT VARIOUS MOISTURE CONTENTS AND AT DIFFERENT INTERVALS FOR A PERIOD OF 28 DAYS

Lab. No.	Kind of Soil	Material added	Amt. material added, gm.	Moisture added		Number of protozoa actually found per 0.33 gm. soil									
						Days									
				Degree opti'um	c.c.	1st	2nd	3rd	4th	6th	8th	10th	14th	21st	28th
21	Sassafras	Nothing	....	¾	13.95	....	....	2 F.[1]	....	....	....	....	....	....	....
22	"	"	....	1	18.60	....	....	....	1 F.	....	....	....	1 S.C.	....	....
23	"	"	....	1½	27.90	....	....	1 F. 1 S.C.	1 F.	....	....	....	1 S.C.	....	....
24	"	"	....	1¾	32.55	....	1 F.	....	2 F. 2 S.C.	1 F.	....	1 S.C.	1 S.C.	3 S.C. 1 L.C	1 F. 1 S.C.
31	"	Dried blood	2.85	¾	16.08	....	....	1 F.	2 F.	1 F.	....	....	....	....	....
32	"	"	2.85	1	21.45	....	....	2 F.	1 S.C.	1 F.	....	1 F.	....	....	....
33	"	"	2.85	1½	30.75	....	1 F. 1 S.C.	2 F. 1 S.C.	....	1 F. 1 S.C.	1 F. 1 S.C.	18 F.	....	....	....
34	"	"	2.85	1¾	35.40	....	4 F.	3 F.	1 F.	3 F. 3 S.C.	....	....	1 F.	1 S.C.	1 S.C.
41	"	Cow manure	2.85	¾	16.62	....	2 F.	3 F.	1 F.	2 F.	....	....	....	....	....
42	"	"	2.85	1	22.16	2 F.	3 F.	5 F.	2 F. 1 S.C.	1 F.	....	....	1 F.	....	....
43	"	"	2.85	1½	33 24	1 F.	1 F.	3 F.	9 F. 2 S.C.	1 F.	1 F. 4 S.C.	2 F. 5 S.C. 4 L.C.	....	....	1 F.
44	"	"	2.85	1¾	38.78	2 F.	1 F.	6 F. 1 S.C.	16 F. 3 S.C.	2 F.	....	1 F.	....	....	1 F. 1 S.C.
52	"	Sand	30	¾	12.07	....	....	....	....	....	....	2 F.	....	1 F.	....
62	"	"	30	1	16.10	....	....	....	....	....	....	....	....	....	....
72	"	"	30	1½	24.15	....	....	....	2 F.	....	1 S.C.	1 F.	1 F.	....	....
82	"	"	30	1¾	28.17	....	....	....	3 S.C.	3 F.	3 S.C.	1 F. 2 S.C.	1 F. 1 S.C.	1 S.C.	....

[1] F.—Flagellates. S. C.—Small Ciliates. L. C.—Large Ciliates.

mined by means of feeling the soil. To each of four 100-gm. quantities of soil, seived air dry, 2.85 gm. of dried blood (representing 50 tons per acre on the basis of 2,700,000 pounds per acre-foot) were added. To another series of 4 tumblers, 2.85 gm. of dry manure, which had been seived through a 20-mesh seive, were added. Into each of 4 tumblers, 30-gm. quantities of pure washed quartz sand which passed a 40-mesh seive were weighed. Then 70 gm. of Sassafras soil were added to each portion. These tumblers containing the mixture of sand and soil, represented the third series. A fourth series of 4 tumblers with 100-gm. portions of soil received no treatment. Each of the soils with the several treatments was then thoroughly mixed by shaking the mixture in a small hard milk-shaking cup[1]. Then to each series, sterile water was added to an amount, making the content of one quantity 30 per cent of the water holding capacity, or three-fourths of the physical optimum, a second received sufficient to bring the moisture to 40 per cent of the water-holding capacity or one optimum, a third 60 per cent, and a fourth 70 per cent of the water-holding capacity. The soil was very carefully mixed so that the moisture would be as evenly distributed throughout the mass as possible. This was accomplished by emptying the contents of the tumblers on a large sheet of manila paper and mixing the material by passing it through the hands several times. Having replaced the contents in each tumbler and weighed the same, they were allowed to incubate at room temperature for a period of 28 days. Examinations of these soils for active protozoa were made by the method given above (three to four 5-minute examinations of each with the high and the low power of the microscope) on the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th, 14th, 21st and 28th days after moistening the mixture. Before each examination was made the tumbler with its contents was carefully weighed and the moisture loss due to evaporation was restored. Then after the examination was complete, the sample was again weighed. By this method the amount of soil used for each examination was easily determined. Hence by counting the number of protozoa actually found in the examination, as was done, the number of active organisms (protozoa) per gram of soil could thus be calculated.

From examining Table I, it is apparent that the period of excystation of protozoa varies with the treatment of the soil, as in all cases where the soil received applications of cow manure the protozoa excysted sooner than they did in the other treatments. In all but one case, where the soil plus manure had a moisture content of three-fourths of the optimum, they were present in the active state on the first day after inoculation, while with no other treatments were they present on this day. Many more active protozoa were counted in the mixtures which received or-

[1] This was recommended by H. C. Lint. See "Sources of Error in Soil Bacteriological Analysis" by Lint, H. C., and Coleman, D. A., p. 157.

ganic matter than where none was applied, likewise, the numbers were greatest in those to which the greatest amounts of moisture were added.

Increasing the porosity of this soil by means of sand did not seem to increase the number of active protozoa present, as there were less noted in the mixture of soil and sand than in the samples of soil alone.

It is noted in this problem, as was very apparent in the development of protozoa in cultural solutions (6), that the flagellates excyst first, the maximum number being present on the 3rd, 4th and 5th days. "At all temperatures, the flagellates develop sooner than the ciliates" (6, p. 557). It would thus appear that it requires a longer period of time for protozoa to excyst than is generally thought. Again, the active flagellates become less in number after the 6th day, when the ciliates become more numerous, so that on the 21st day only one small flagellate was noted in one soil while ciliates were found to be present in three. It is very plain that after a certain period of time the active protozoa decrease in number very rapidly, so that even though the moisture conditions would be considered very favorable and also an abundance of organic matter would be present, after a time the protozoan population in the active state would be almost *nil*. This above-mentioned fact no doubt explains the questions which Waksman (19, p. 136) raised in regard to the author's previous work[1] when he said, "The error made in limiting the time of examination to only 2 minutes is made clear by the fact that out of 20 greenhouse soils, where Koch found protozoa, the organisms recorded are in 5 cases ciliates and amoebae, and only in one case flagellates, and further as was shown by Sherman (16), Cunningham (2), by the writer and others, the number of flagellates is much larger than the number of ciliates, and we would expect that under conditions when moisture is the limiting factor, the smaller organisms would be able to lead an active life at a lower moisture content than the larger ones." It would seem, as shown above, that after a certain time and stage of decomposition of organic matter, active flagellates cease to be present to any great extent while living ciliates are more numerous. No doubt if the greenhouse soils to which Waksman refers had been examined soon after they were composted, the active flagellates would have out-numbered the ciliates considerably.

The question may be asked why more active protozoa were not found in the laboratory studies with the three soils in the previous work (7). This is explained by the fact that two of these soils were of close texture (heavy soils) one being a shale compost and the other a clay loam. It will be seen later that with a heavy soil (Penn clay loam) very few active protozoa are found. The first of these contained organic matter but as it had reached an advanced stage of decomposition it would not influence protozoan development to a very great extent. One small cili-

[1] It is presumed that Waksman refers to Koch's (7) "Activity of Soil Protozoa."

ate was noted on the third day in the case of the field soil containing one and one-half optimum moisture. None of these soils received any organic matter in the laboratory treatments. No doubt their absence in the air-dried samples and those containing only one-half of the optimum of moisture was due to the lack of moisture. Furthermore even with a 15 to 20-minute examination, but very few (4 organisms) were found to be present in the examinations up to and including the 8th day of the present work, as shown in Table I (soil samples with one optimum and one and one-half optimums of moisture and receiving no organic matter, No. 22 and No. 23).

In order to demonstrate that the writer did not err in his 2-minute examination for 30 minutes in his previous work (7) as was questioned in the quotation above, after having found active protozoa to be present on the second day in this experiment, he concluded that certainly some would be present in the active condition on the third day. Hence to check up the 2-minute examination period, the length of time required to find a living protozoan was recorded on the third day. After the protozoa had been found, a different sample was taken and the examination of each soil was continued for a period of 20 minutes.

By noting the time required to find the active protozoa in the examination, it is seen that there are but few cases where these organisms were not found within the 2-minute period. Moreover, the samples of field and of greenhouse soil which were examined in the above referred work, were under observation for a 30-minute period, in which the experimenter is very liable to find the organisms. It is true that there were a comparatively large number of active protozoa present in the samples to which organic matter (dried blood and cow manure) had been added when this examination was made, but it will be noted that they were also found in the specified time (2 minutes) in the samples which received no organic matter, where the numbers were very low.

It might be stated that about 0.33 gm. of soil represented the amount taken for each daily examination for active protozoa, hence by multiplying by three the number of active protozoa recorded, found in Table I and in Table III, one could obtain a fairly good representation of the number of motile protozoa present per gram of soil. It would hardly seem that 0.33 gm. would be an adequate representative amount of soil upon which to base conclusions, but by this method (direct examination) it is, not possible to examine between the particles of more than this amount in 3 or 4 examinations. Moreover, in bacteriological technique, it is a common practice to pour plates from dilutions, which represent 1-10,000 and 1-50,000 of a gram. The largest number of flagellates found in one day's examination of a single sample was 18. This would mean that there were approximately 54 flagellates per gram of soil, which may be compared with at least 100,000,000 to 300,000,000 or more bacteria per gram of soil, as shown later.

Having found that soil protozoa excyst for only a short period of time and that active flagellates appear first, followed by the ciliates and that the number of motile protozoa relative to bacteria is comparatively small, it was next desired to repeat the same experiment with a different soil.

TABLE II

THE LENGTH OF TIME REQUIRED TO FIND ACTIVE PROTOZOA AND THE NUMBERS FOUND BY THE DIRECT METHOD IN THE EXAMINATION OF THE VARIOUS TREATMENTS OF SASSAFRAS SOIL

Lab. No.	Organisms found in 20 minutes	Length of time to find organism min.-sec.
21	1 F.[1]	1:56
21	1 F.	3:10
22	none	....
23	1 F.	4:45
23	1 S.C.	1:46
24	none	....
31	1 F.	2:30
32	1 F.	1:40
	1 F.	0:30
33	1 F.	2:00
	1 F.	1:50
	1 S.C.	0:31
34	1 F.	1:00
	1 F.	0:26
	1 F.	0:40
41	1 F.	1:20
	1 F.	1:13
	1 F.	2:00
42	1 F.	2:50
	1 F.	3:10
	1 F.	2:05
42	1 F.	1:30
	1 S.C.	0:30
43	1 F.	2:28
	1 F.	0:32
	1 F.	1:19
44	1 F.	0:36
	1 F.	1:48
	1 F.	0:33
44	1 F.	1:03
	1 F.	0:09
44	1 F.	1:04
	1 S.C.	0:19
52	none	....
62	none	....
72	none	....
82	none	....

[1] F.—Flagellate. S. C.—Small Ciliates.

Hence a typical Penn clay loam was used. Inasmuch as active protozoa were found in all the samples of the Sassafras soil, even with lowest (three-fourths of the optimum) moisture content, in repeating the above problem with the Penn loam soil in each series, an additional quantity of soil was taken and moisture to the amount of one-third of the optimum was added.

Likewise, the experiment was repeated with the Sassafras soil under the same conditions as in the preceding experiment, except that the moisture content was one-third of the optimum. Careful examinations for a period of 15 to 20 minutes were made of each sample on the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 14th days.

Upon noting the upper portion of Table III and of Table I, it will be seen that the data indicate that with this soil (Sassafras) the moisture is the limiting factor which determines the presence of the protozoa in the active condition, while the organic matter and sand (porosity) added are secondary factors, a fact which did not hold for bacteria, in that some types of bacteria were always present at this (one-third of the optimum) moisture content. Again, with the Penn clay loam soil the greater number of protozoa became active on the 2nd, 3rd and 4th days, after which the number decreased so that there were practically none noted after the 6th day. With this soil, the number of active protozoa was very small as compared with the numbers counted in the previous problem (Table I) with the Sassafras soil. In many cases they were not found at all on any day, even where there was as much as 50 tons of cow manure per acre present, and with moisture to the amount of one and one-half optimum. With this in mind, it would hardly seem that they would play an important rôle in soil biological processes if their presence in the active state as determined by these methods, is used as an index. Apparently this type of soil (Penn loam) seemed to be a very unfavorable medium for active protozoa. A very small number might have excysted the second or third day, and so few being active for such a limited period of time, they might already have become inactive when the examinations were carried out. Dried blood seemed to favor their development to some extent. In sample No. 35, with the Penn soil containing 2.85 gm. of dried blood and a moisture content of one-third of the optimum, one small flagellate was seen to be present on the second day. This might have been due to the fact that the soil moisture was not equally distributed throughout the mixture as it is very difficult to get the moisture evenly distribued throughout the mass especially where such a small amount is added.

The addition of a sand to the soils increasing the porosity and aeration did not seem to encourage the increase of active protozoa.

In order to secure more data upon the effect of the addition of organic matter upon the presence of protozoa in the active condition, sample No. 22 with optimum moisture as shown in Table I, which had been under examination until after the 28th day, was employed for this work. In like manner, tumbler No. 20, which contained Sassafras soil with a moisture content of one-third optimum and tumblers No. 25 and No. 27 with Penn soil having moisture of one-third optimum and optimum, respectively, and which had been examined for 14 days were also used. To each of these soils, 2.85 gm. of dried blood were added and carefully

TABLE III

PRESENCE OF ACTIVE PROTOZOA IN A SASSAFRAS SOIL AND PENN CLAY LOAM, WHICH HAD RECEIVED TREATMENTS OF DRIED BLOOD AND COW MANURE, AT VARIOUS MOISTURE CONTENTS AND AT DIFFERENT INTERVALS FOR A PERIOD OF 14 DAYS

Lab. No.	Kind of Soil	Material added	Amount of material added	Moisture added		Number of protozoa actually found after inoculation per 0.33 gm. soil							
						Days							
				Degree opt'um	c.c.	1st	2nd	3rd	4th	6th	8th	10th	14th
20	Sassafras	Nothing	....	1/3	6.20	.....	.....	.....	.....	.....	.....	.....	.....
30	"	Dried blood	2.85	1/3	7.15	.....	.....	.....	.....	.....	.....	.....	.....
40	"	Cow manure	2.85	1/3	7.38	.....	.....	.....	.....	.....	.....	.....	.....
50	"	Sand	30	1/3	5.37	.....	.....	.....	.....	.....	.....	.....	.....
25	Penn	Nothing	....	1/3	8.[illegible]	.....	.....	.....	.....	.....	.....	.....	.....
26	"	"	....	¾	18.42	.....	.....	.....	.....	.....	.....	.....	.....
27	"	"	....	1	24.56	.....	.....	.....	.....	.....	.....	.....	.....
28	"	"	....	1½	36.84	.....	.....	.....	.....	.....	.....	.....	.....
29	"	"	....	1¾	42.98	.....	2 F.[1]	.....	1 F.	.....	.....	.....[illegible]	.....
35	"	Dried blood	2.85	1/3	9.14	.....	1 F.	.....	.....	.....	.....	.....	.....
36	"	"	2.85	¾	20.55	.....	1 F.	.....	.....	.....	.....	.....	.....
37	"	"	2.85	1	27.41	.....	.....	.....	.....	.....	.....	.....	.....
38	"	"	2.85	1½	39.69	.....	1 F. 1 S.C.	.....	.....	1 F.	.....	.....	.....
39	"	"	2.85	1¾	45.83	.....	1 F.	1 F.	1 S.C.	.....	.....	.....	.....
45	"	Cow manure	2.85	1/3	8.72	.....	.....	.....	.....	.....	.....	.....	.....
46	"	"	2.85	¾	19.62	.....	.....	.....	.....	.....	.....	.....	.....
47	"	"	2.85	1	26.16	.....	.....	.....	.....	.....	.....	.....	.....
48	"	"	2.85	1½	39.24	.....	.....	.....	.....	.....	.....	.....	.....
49	"	"	2.85	1¾	45.78	.....	1 F.	1 F. 1 S.C.	.....	.....	.....	.....	.....
53	"	Sand	2.85	1/3	6.15	.....	.....	.....	.....	.....	.....	.....	.....
54	"	"	2.85	¾	13.83	.....	.....	.....	.....	.....	.....	.....	.....
64	"	"	2.85	1	18.44	.....	.....	.....	.....	.....	.....	.....	.....
74	"	"	2.85	1½	27.66	.....	1 S.C.	.....	1 F.	.....	.....	.....	.....
84	"	"	2.85	1¾	32.27	.....	.....	.....	.....	.....	.....	.....	.....

[1] F.—Flagellates. S. C.—Small Ciliates.

mixed as before. These samples were examined for living protozoa on the 1st, 2nd, 3rd, 4th, 6th, 8th and 14th days.

TABLE IV

PRESENCE OF ACTIVE PROTOZOA IN SOILS, WITH DIFFERENT MOISTURE CONTENTS, WHICH HAD PREVIOUSLY BEEN INCUBATED FOR A DEFINITE LENGTH OF TIME AND WHICH LATER RECEIVED ORGANIC MATTER

Lab. No.	Kind of Soil	Moisture during first incubation c.c.	Moisture after Dried Blood was added c.c.	Presence of active protozoa before Dried Blood was added	Presence of protozoa after inoculation							
					Days							
					1st	2nd	3rd	4th	6th	8th	10th	14th
20	Sass.	6.20	7.15	....	....	....	....	....	....	....	....	....
22	Sass.	18.60	21.45	....	....	....	....	....	....	....	....	....
25	Penn	8.19	9.14	....	....	....	....	....	....	....	....	....
27	Penn	24.56	27.41	....	....	....	....	....	....	....	....	....

From the above data it is seen that not only did the protozoa fail to excyst in the soils with a low moisture content, but they were not even noted in the soils which had an optimum moisture content. This might be explained by the fact that during the previous incubation of these soils, before the dried blood had been added, the organisms (bacteria, actinomyces, fungi, and in some cases protozoa, No. 22) multiplied, and decomposing more or less of the substances used for life processes, left products of assimilation behind which no doubt were unfavorable to their presence. Hence they did not appear in the sample even where the conditions were favorable for their development before. This shows that there is still another factor, other than moisture, presence of organic matter, physical property of the soil and temperature, which determines the presence or absence of active protozoa.

It might be stated that the inconsistency (as termed by Waksman) of Koch's (7) former results with the greenhouse soils, in that in some cases, active protozoa were found to be present at a lower moisture content than at others, and that Koch stated that the moisture was the primary limiting factor, can be explained as follows: By noting in the table the extent of protozoan activity in greenhouse soils (7) it will be seen that "no mixtures" and "20 per cent compost plus sand" contained the lowest percentage of moisture while the 40 per cent composts contained the highest. Thus when active protozoa were not found in soils with 34 to 36 per cent moisture, one of the reasons might have been that the actual free water at such a high compost was less than in a "no mixture" containing 22.59 per cent of moisture where motile protozoa were found.

The data as presented in Table I and II point to the fact that the greatest number of protozoa became active on the 2nd, 3rd, 4th and 6th

TABLE V

PRESENCE OF ACTIVE PROTOZOA AND NUMBERS OF BACTERIA IN SAMPLES OF SASSAFRAS AND PENN CLAY LOAM SOILS WITH AN ADDITION OF DRIED BLOOD, AT DIFFERENT MOISTURE CONTENTS AND AT VARIOUS INTERVALS FOR A PERIOD OF 14 DAYS

Lab. No.	Kind of Soil	Moisture added c.c.	Days																								
			1st			2nd			3rd			4th			6th			8th			10th			14th		
			Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.
				15			44			104			*			115			168			174			*	
101	Sass.	7.15	—	11	12.0	—	54	50.0	—	90	101.6	—	132	117.5	—	108	112.0	—	120	150.0	—	168	148.0	—	36	38.0
				10			52			111			103			113			162			102			40	
				8			46			66			122			90			114			180			23	
102	"	7.15	—	11	9.6	—	39	45.0	—	103	86.6	—	102	112.0	—	90	98.0	—	108	100.0	—	90	118.0	—	29	24.6
				10			50			91			112			114			78			84			22	
				16			132			126			138			330			540			18			30	
103	"	21.45	—	18	17.6	—	96	108.0	1 F.	114	140.0	1 F. 1 S.C.	186	172.0	1 F.	242	275.0	1 S.C.	570	564.0	5 F.	12	12.0	—	28	26.6
				19			96			180			192			253			582			6			22	
				26			132			78			126			99			294			306			14	
104	"	21.45	—	16	17.0	—	162	140.0	1 F.	66	82.0	1 F.	120	116.0	—	231	183.3	—	342	314.0	1 F.	396	338.0	—	18	14.6
				9			126			102			102			220			306			312			12	
				20			33			29			29			42			48			6			43	
201	Penn	9.14	—	19	18.0	—	27	28.0	—	32	28.3	—	25	28.0	—	24	34.0	—	30	32.0	—	—	8.0	—	39	37.3
				15			24			24			30			36			18			18			30	
				22			32			29			25			24			30			12			38	
202	"	9.14	—	14	17.3	—	31	30.6	—	34	33.3	—	32	27.6	—	30	30.0	—	36	32.0	—	6	10.0	—	31	36.0
				16			29			37			26			36			30			12			39	
				64			108			36			102			66			48			30			90	
203	"	27.41	—	74	67.0	—	84	86.0	—	48	48.0	—	66	84.0	—	38	49.3	—	72	66.0	—	36	26.0	—	53	69.0
				63			66			60			84			44			78			12			64	
				53			72			96			66			33			174			90			102	
204	"	27.41	—	54	56.3	—	72	82.0	—	49	64.0	—	36	52.0	—	44	44.0	—	108	126.0	—	78	76.0	—	113	106.3
				62			102			47			54			55			96			60			104	

NOTE:—Numbers of bacteria represent millions per gram of soil. Numbers of protozoa are for 0.33 gm. of soil.

* Lost.

days. It was now desired to ascertain if during this period there would be a marked decrease in the number of bacteria. The possible destructive character of protozoa upon bacteria seemed to be the best index of the effects of protozoa. In the case where no protozoa were seen to be present, as at one-third of the optimum moisture content, one might infer that up to a certain point the bacterial multiplication would be consistent, while where the protozoa were seen to be present, their multiplication (bacteria) would be irregular. With this in mind four 200-gm. quantities of the same Sassafras loam and like quantities of Penn clay loam which had previously been used were weighed out into tumblers. Each tumbler received 5.70 gm. of dried blood, (or the equivalent of 50 tons per acre). Sufficient sterile water was added to two tumblers of the Sassafras series and two of the Penn series to make the soil plus dried blood of one-third of the optimum moisture content. In like manner two tumblers of each soil plus dried blood received sufficient water to bring the moisture content to optimum. These were carefully mixed as before and then placed in the incubator. Careful examinations of each tumbler for active protozoa for a period of 15 to 20 minutes were made from the 1st to the 14th days (inclusive) as before. The data were recorded each day. After the examinations had been completed 10 gm. were weighed out of each of the tumblers and this was used for making the bacteria counts[1].

Upon noting Table V it is seen that these data (part showing the presence of active protozoa) corroborate that which is shown in Tables I and III with these soils and the corresponding moisture content. Active protozoa were first seen to appear on the third day in the Sassafras soils containing the optimum amount of moisture. They were noted every day thereafter until and including the 10th with one sample, while with the other they were not seen on the 6th or 8th days. In the samples containing the lower amounts of moisture (one-third optimum) they were never seen to be present. Motile protozoa were never observed in any of the samples of the Penn clay loam soil. If they were active at any time in the samples of this soil they were either so minute that they could not be distinguished or the numbers were so very small that a 15 to 20 minute examination did not show any.

Is there any correlation between the bacterial numbers and the presence of active protozoa as determined by the direct examination? Glancing through Table V, which shows the number of bacteria present in the various samples of soil, it is seen that with samples No. 101 and No. 102, there is a more or less gradual increase in numbers of bacteria up to and

[1] The bacterial counts of each tumbler were made in triplicate on Lipman and Brown's "synthetic" agar. Lipman, J. G., and Brown, P. E. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt., p. 129-136. 1908.

including the 10th day, and a very decided decrease on the 14th day. As stated before and shown in Table V, active protozoa were never seen to be present in either of these samples. In samples No. 103, there is a marked increase in bacterial numbers until the 8th day even though active protozoa were observed on the 4th, 6th and 8th days. On the 10th day there is a very marked decrease in bacteria and a greater number of protozoa were also counted on this day. In the case of sample No. 104, this increase in bacterial numbers from day to day is not so consistent as it is with No. 103 (the duplicate of 104), but even in the latter sample, we do not find any reduction in bacteria after the third day until the 14th even though motile protozoa were observed. If the active protozoa in No. 103 were effective in destroying bacteria, then the multiplication of bacteria must have been much greater than that in sample No. 104 in order that the greater number of bacteria in No. 103 can be accounted for, as more protozoa were also noted in this latter sample than in No. 104; or else the motile protozoa did not affect the numbers of bacteria to any appreciable extent, since it is noted that in samples Nos. 101 and 102, where the moisture content was low, there was a great decrease of bacterial numbers on the 14th day and yet living protozoa were never seen. Again, on the 14th day, in samples Nos. 103 and 104, we find a great decrease in bacterial numbers. Is this due to the effect of the active protozoa which were seen to be present on the 10th day? If this is true, it would agree with Russell (11) in his objection that the effect of the detrimental factor cannot be seen in a 7-day incubation period; hence the protozoa which had been present in the active condition might not have been of the destructive type. Again, might the decrease in the numbers of bacteria have been due to other causes, as the presence of fungi or other organisms, or to the presence of toxic decomposition products?

Upon examining the results, with the Penn soil, it is seen that in the samples containing moisture of 1/3 optimum, the bacterial numbers were practically constant until the 10th day. On this day a marked decrease was noted and then a great increase on the 14th day. It would hardly seem possible that this decrease in bacterial numbers was due to a detrimental factor (namely protozoa) as they were never observed to be present in the motile condition. The irregularity in numbers of bacteria was noted in a piece of work by the writer (8) where he counted organisms (*B. coli.*) developed from day to day over a period of time. Just such a cause may be due to the variation in the numbers of bacteria which were observed in this problem. Again in Nos. 203 and 204 there is a fluctuation in bacterial numbers from day to day and still no protozoa were ever noted to be present.

It is true that an investigator could hardly conclude that the results of such a problem as above, where all the organisms of the soil as well as the other conditions as moisture, assimilation products, etc., must be considered, are due to the one specific factor with which he is working. However, the intention of this work, as in the case of the previous work (7), is to study normal soils as they occur and thus see whether or not protozoa if they are a factor, exist in the normal conditions (that is, as nearly normal as possible in carrying out laboratory experiments) and to correlate if possible the factors studied (bacteria and protozoa) under these conditions.

Can the results obtained by employing partially sterilized sewage-sick soils or partially sterile normal soils, in which cases only some of the factors are eliminated, be used to demonstrate the effects of a detrimental factor (probably protozoa) applied to normal agricultural soils? As shown recently by Coleman, Lint and Kopeloff (1), partial sterilization of normal soils destroys only some of the factors which function in soil fertility, and it greatly influences (alters) the character of the soil as a medium.

No doubt, under very abnormal conditions (as regards agricultural practices), for instance in the case of sewage-sick soils which contain a great abundance of organic matter and whose floral population is entirely different from that of soils under tillage, containing not only an abundance of protozoa but also eel worms, vermes and many other living forms, and under other conditions, as aeration, etc., entirely different from those under which the soil is tilled normally, a limiting factor might be of immense importance.

In the light of the results of this paper and of the paper cited above (7), it would seem that in a practical way, protozoa are of slight importance in limiting the bacterial flora of the soil, as their presence in the active state is very limited. In no cases were they found to be present in the active state in the field soils examined, in only 6 out of the 20 greenhouse soils were protozoa found, and in these soils their numbers were very limited. The data as given in this paper would indicate their presence in some soils to which a large amount of organic matter (50 tons per acre) has been applied, but even under these conditions of fertility, when more organic matter is applied than is ever used under ordinary field agricultural methods, they are active only to a very limited extent, the numbers decreasing decidedly 8 or 10 days after the organic matter has been added. Moisture seems to be the primary factor which in these soils and others, determines their presence in the active condition, as active protozoa were never noted in soils which were air-dry or had a very low moisture content, even though there was an abundance of organic matter and the texture was of very open character. This is not true of bacteria however, as some are in the active condition in air-dried soils.

Separation of Bacteria from Protozoa

Under certain conditions, in carrying out soil biological experiments in the laboratory and eventually in the field (9) for a period of 7 to 10 days, we have protozoa present in the active condition. Are they an influential factor in the biological method as now employed? Considering the very small numbers which are present in the active condition as compared with bacteria, and the fact that they are present for such a short period of time, we would hardly think they would be a factor. However, in desiring to prove this point, the author is attempting to get a protozoa culture free from bacteria. He plans to inoculate the medium with these clean cultures and in this way note their effect upon bacterial activities.

For this work a special centrifuging tube was devised by the writer and has been proving satisfactory although a complete separation of bacteria from protozoa has not yet been accomplished.

The apparatus, as pictured in figure 1, consists of a glass centrifuging burette, 10½ inches long and having a capacity of 6 c.c. This special burette has two stop cocks at its lower extremity. The contents of the small tube between the stop cocks is about 1 c.c.

The protozoa which are much heavier than bacteria are forced down by gravity in the centrifuging process as has been previously tried by Russell and Golding (13) and later by Jones (5).

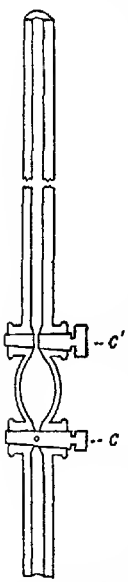

Fig. 1—Diagram of apparatus used for separating protozoa from bacteria.[1]

The procedure of this method is as follows: Cock C being closed and cock C' open and the lower end (delivery tube) of the burette having been plugged with cotton, 5 c.c. of the medium containing protozoa and bacteria are introduced into the sterile apparatus. The apparatus with contents is now placed into the centrifuge and a certain number of revolutions are made. The protozoa are thus forced down through the opening of cock C' and are held in the small tube between C and C'. Cock C' is closed and the solution in the upper part of the burette which contains the greater number of bacteria is poured out. This section of the apparatus is now sterilized with alcohol and then some of the sterile medium is introduced. Cock C' is now opened and the protozoa which are held in the tube between C and C' are washed into this sterile medium. Centrifuging is repeated, cock C' closed and the same process repeated as before. In this manner the protozoa can be washed several times until the bacteria are all washed from them. The protozoa having thus been freed from the bacteria, cock C is opened and the protozoa are delivered into the desired container by means of the small delivery tube at the lower extremity of the burette.

[1] The author is indebted to Dr. J. W. Shive for preparing this drawing.

SUMMARY

Under the conditions with which the problems recorded in this paper were carried out, the following results as to protozoan activity in the soils have been noted:

1. The direct examination of the soil under the microscope for a period of 15 to 20 minutes (taking 3 or 4 samples), gives a fair indication as to the relative extent of active protozoa in the soil.

2. Practically all of the protozoa as found in the soil can be observed under the low power of the microscope by the careful experimenter.

3. The addition of organic matter to the Sassafras sandy loam soil encouraged a greater protozoan development than where none had been added.

4. Additions of dried blood to the Penn clay loam soil increased the number of active protozoa but very little while cow manure had no effect on the protozoan activity of this soil.

5. Increasing the porosity and aeration of the soils by the addition of sand did not increase the number of motile protozoa.

6. In the Sassafras soil to which cow manure had been added, flagellates were noted on the 1st day, while with the other samples they were not seen until the 2nd or 3rd and in some cases the 4th day. The maximum numbers were recorded on the 4th and 6th days after which there was a marked decrease so that on the 21st and 28th days practically none were found to be in the active state. This would indicate that the destructive ability, (if soil protozoa possesses that factor) would be present for only a limited period, namely the early stages of organic decomposition.

7. Flagellates were the first protozoa to excyst, later, on the 3rd and 4th days, small ciliates appeared. On the 14th, 21st and 28th days, small ciliates were more numerous than flagellates.

8. With these soils, the largest number of protozoa was recorded where the moisture was highest.

9. The Penn clay loam soil seemed to be a very undesirable medium for protozoa, as very few of these organisms were noted in any of the samples compared with those found in the Sassafras soil.

10. At the lowest moisture content (one-third of the optimum) protozoa did not become active.

11. With these soils, moisture seemed to be the primary limiting factor which determined the presence or absence of active protozoa.

12. With one exception, no correlation between the presence of protozoa in the active condition and numbers of bacteria could be seen. Increased numbers of bacteria were observed irrespective of the presence or absence of living protozoa.

13. On the 14th day in the soils, even where no protozoa were found in the active condition, there was a great decrease in bacterial numbers.

14. Inasmuch as the numbers of protozoa in comparison with the bacterial numbers are so small, even in the presence of such abnormal quantities of organic matter as were used in these experiments, it hardly seems that they would be of very great importance in agricultural practices.

It has been previously shown (7):

1. Under ordinary greenhouse conditions, small ciliates, flagellates and amoebae are active in some soils, but their presence is very limited.

2. Active protozoa (small ciliates, large ciliates, flagellates, and amoebae) do not seem to be present in field soils with a normal moisture content, and even when the moisture content is slightly supernormal; hence, they would not be a limiting factor in the soil.

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AZOTOBACTER IN HAWAIIAN SOILS[1]

By

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Since those reading this brief paper are doubtless familiar with the aerobic, non-symbiotic species of nitrogen fixing bacteria termed Azotobacter, a detailed discussion of the properties of this class of microorganisms together with a review of its extremely copious literature would here be both objectionable and superfluous.

While working in the laboratories of Soil Chemistry and Bacteriology at the California Agricultural Experiment Station some time ago, many samples of soil from different parts of the world were examined by the writer for Azotobacter species (1). This work was done under the direction of Dr. C. B. Lipman and with the object of ascertaining the universality of distribution of this most interesting group of soil organisms. We were especially fortunate in having at our disposal the large and unusually complete soil collection of the late Dr. E. W. Hilgard, from which about fifty soils, from widely separated parts of the world, were chosen for the work. These included some six or eight samples of Hawaiian soils, and it may be added that later about a dozen more samples from the Hawaiian Islands were further investigated. With but one or two possible exceptions, no Azotobacter species were discovered in the Hawaiian soils examined. Were these dry soil samples too old to retain this species still in a living condition even had they been originally present, or had Azotobacter not yet become generally distributed in the soils of these isolated islands which, geologically speaking, are of very recent origin? These were questions which, at the time we were unable to answer, but which now furnish an excuse for the publication of this paper.

About six months ago it fell to the writer's lot to visit the four large islands of the Hawaiian group and to collect a large number of soil samples from the several typical soil areas there found. These samples were drawn for another purpose, but, remembering his previous failure to isolate Azotobacter from similar soils, several fresh samples from each of the islands of Oahu, Hawaii, Maui and Kauai were studied as to their

[1] Received for publication June 20, 1916.

Azotobacter content. So far as the writer is aware, nothing has appeared concerning the forms or physiological activities of Azotobacter species isolated from Hawaiian soils.

The samples were all taken from cultivated fields which at the time were carrying heavy crops of sugar cane.

Experimental

The soil samples were all received in new, heavy-canvas, sterilized bags, each of which held about ten pounds of soil. A sterilized (in flame) trowel was used in taking the samples. As soon as a bag was filled it was closed and labeled. These small bags were placed in large sugar bags and shipped directly to the laboratory in Honolulu. When received, the different samples were carefully spread out on sterile (washed with alcohol) pieces of oil-cloth about 2 feet square, in the basement, and allowed to air-dry. The samples were then passed through sterile 2-mm. sieves. The soils were then replaced in their original bags and so kept in a large cold chamber until used. Over one hundred samples of surface soils were so collected and treated, together with their subsoils. Only thirty surface soils were used in the work here reported, however.

Table I gives the laboratory number and the localities from whence each of the different soil samples came.

In selecting these samples practically all of the different arable soil types of any considerable area encountered here on the islands have been covered. Where two samples were taken from the same plantation, the first was drawn from the low lands near the sea, while the other came from the higher fields (from 700 to 1200 feet above sea level).

I have on hand analytical data covering all of the above samples, but they find no place in this paper. It may be stated, however, that all of the soils listed above, with the exception of two or three coral formations, are laterite soils derived from basaltic lavas, and contain from 40 to 65 per cent combined oxides of iron and aluminum, "Hilgard Method of Analysis." Physically, most of the soils above listed would be classed as silty, clay loams of high organic matter content, the percentage of total nitrogen varying from 0.2 per cent to over 1.00 per cent. Most of the soils are of a dark brown or chocolate color, although there are some exceptions. Those from the Oahu Sugar Co. are a very bright brick red, while those from Honolulu Plantation are a light drab or gray.

The nutrient solution employed was made up as follows:

In 1000 c.c. tap water was dissolved:

0.2 gm. K_2HPO_4
0.2 gm. $MgSO_4$
0.5 gm. NaCl
20.0 gm. mannite
1 drop of a 10 per cent $FeCl_3$ solution.

A few drops of phenolphthalein were then added to the solution and it was rendered slightly alkaline with a 5 per cent solution of KOH. One-hundred-c.c. portions of this solution were placed in 500-c.c. Jena Erlenmeyer flasks, plugged with cotton, and sterilized at 10 pounds pressure for 15 minutes. Each of these flasks, cooled, was then inoculated with exactly 1 gm. of the air-dried soil under investigation, the nitrogen contained therein being finally subtracted from the total fixation as shown in Table III.

TABLE I

LOCALITIES FROM WHENCE SOIL SAMPLES WERE TAKEN

Laboratory No.	Island	Plantation
1	Hawaii	Waiakea Mill Co.
2	Hawaii	Waiakea Mill Co.
3	Hawaii	Onomea Sugar Co.
4	Hawaii	Onomea Sugar Co.
5	Hawaii	Hawaiian Agricultural Co.
6	Hawaii	Hawaiian Agricultural Co.
7	Hawaii	Pacific Sugar Mill.
8	Hawaii	Pacific Sugar Mill.
9	Hawaii	Honokaa Sugar Co.
10	Hawaii	Honokaa Sugar Co.
11	Hawaii	Hamakua Mill Co.
12	Hawaii	Hamakua Mill Co.
13	Hawaii	Kaiwiki Sugar Co.
14	Hawaii	Kaiwiki Sugar Co.
15	Hawaii	Pepeekeo Sugar Co.
16	Hawaii	Kukaiau Plantation Co.
17	Oahu	Experiment Station, Honolulu.
18	Oahu	Honolulu Plantation Co.
19	Oahu	Honolulu Plantation Co.
20	Oahu	Ewa Plantation Co.
21	Oahu	Waipio Field Experiment Station.
22	Oahu	Oahu Sugar Co.
23	Oahu	Oahu Sugar Co.
24	Maui	Hawaiian Commercial and Sugar Co.
25	Maui	Hawaiian Commercial and Sugar Co.
26	Maui	Pioneer Mill Co.
27	Maui	Pioneer Mill Co.
28	Kauai	McBryde Sugar Co.
29	Kauai	McBryde Sugar Co.
30	Kauai	Hawaiian Sugar Co.

These mixed cultures were then incubated 3 weeks at 28° C. At the end of 5 days, beautiful Azotobacter membranes had appeared in many of the flasks. At the end of one week the appearance of these cultures was as follows:

SOIL CULTURE No. 1.

Color of solution	—	gray
Pigment	—	white, glistening
Membrane	—	at edges of flask only
Gas production	—	slight
Turbidity	—	slight
Odor	—	butyric acid, slight

SOIL CULTURE No. 2.
Very similar to No. 1, except that membrane extended over entire surface.

SOIL CULTURE No. 3.

Color of solution	—	colorless
Pigment	—	none
Membrane	—	very thin, at edges
Gas production	—	heavy
Turbidity	—	slight
Odor	—	strong butyric

SOIL CULTURE No. 4.
Similar to No. 3, except that there was no membrane.

SOIL CULTURE No. 5.

Color of solution	—	drab
Pigment	—	heavy, orange-colored
Membrane	—	heavy over entire surface
Gas production	—	medium
Turbidity	—	heavy
Odor	—	butyric

SOIL CULTURE No. 6.

Color of solution	—	gray
Pigment	—	beautiful, white, glistening
Membrane	—	heavy over entire surface
Gas production	—	heavy
Turbidity	—	very turbid
Odor	—	vile, putrescent

This organism is apparently quite widely distributed throughout these island soils, for the description of Soil Culture No. 6 can very well serve for the following soil cultures:—Nos. 8, 10, 12, 16, 23, 26 and 29. It is true that they were not all absolutely identical in appearance, yet their remarkable similarity showed them to contain the same species of Azotobacter. This was verified by microscopical examination.

SOIL CULTURE No. 7.

Color of solution	—	brown
Pigment	—	none
Membrane	—	none
Gas production	—	heavy
Turbidity	—	heavy
Odor	—	strongly butyric

SOIL CULTURE No. 9.
Identical with Soil Culture No. 7.

SOIL CULTURE No. 11.

Color of solution	—	yellow
Pigment	—	golden yellow
Membrane	—	heavy over entire surface
Gas production	—	strong
Turbidity	—	medium
Odor	—	strong, butyric

SOIL CULTURE No. 13.
Description omitted by mistake.

SOIL CULTURE No. 14.
Similar to Soil Culture No. 7.

SOIL CULTURE No. 15.
Identical with Soil Culture No. 11.

SOIL CULTURE No. 17.

Color of solution	—	drab to chocolate color
Pigment	—	black
Membrane	—	over entire surface, heavy
Gas production	—	heavy
Turbidity	—	medium
Odor	—	none

SOIL CULTURE No. 18.

Color of solution	—	light brown
Pigment	—	tan-colored, black spots
Membrane	—	over entire surface
Gas production	—	slight
Turbidity	—	medium
Odor	—	strong, butyric

SOIL CULTURE No. 19.

Color of solution	—	almost colorless
Pigment	—	black with dark orange spots
Membrane	—	heavy over entire surface
Gas production	—	medium
Turbidity	—	medium
Odor	—	damp leaf mold, earthy

SOIL CULTURE No. 20.

Color of solution	—	none
Pigment	—	white, glistening; slightly yellowish
Membrane	—	thick over entire surface
Gas production	—	slight
Turbidity	—	slight
Odor	—	butyric

SOIL CULTURE No. 21.

Color of solution	—	light brown
Pigment	—	smooth, white; black at edges
Membrane	—	very thin over entire surface
Gas production	—	none
Turbidity	—	slight
Odor	—	putrescent

SOIL CULTURE No. 22.

Color of solution	—	reddish
Pigment	—	black with orange spots
Membrane	—	thick, very mottled
Gas production	—	heavy
Turbidity	—	medium
Odor	—	weak butyric

SOIL CULTURE No. 24.

Color of solution	—	yellowish green
Pigment	—	beautiful golden yellow
Membrane	—	over entire surface, heavy
Gas production	—	heavy
Turbidity	—	very turbid
Odor	—	strong butyric

SOIL CULTURE No. 25.

Similar to Soil Culture No. 24 except that here the membrane was very thin and at the edges of the flask only.

SOIL CULTURE No. 27.

No pigment and no membrane. Similar to Soil Culture No. 7.

SOIL CULTURE No. 28.

Very similar to Soil Culture No. 24.

SOIL CULTURE No. 30.

Color of solution	—	brownish
Pigment	—	pale orange-colored with black spots
Membrane	—	thin
Gas production	—	weak
Turbidity	—	slight
Odor	—	weakly putrescent

After recording the appearance of the mixed cultures, all of those showing membranes dissimilar in color or appearance were plated out on mannite agar and the plates incubated for from 4 to 8 days at 28° C. The writer will not burden the reader with a detailed description of these plates. Suffice it to say that, in the end (after several replatings), four different Azotobacter forms were isolated in pure cultures. Two of these were identified as *A. chroococcum* (isolated from Soil Culture No. 18), and *A. Vinelandii* (isolated from Soil Culture No. 24). Of the two remaining unidentified organisms, one was very similar to *A. Vinelandii* in morphology and cultural characteristics, with the following few exceptions. The individual cells are slightly smaller although of similar shape. There is no tendency to form a colored pigment. Even on old mannite agar slants the growth remains white, there being no yellow or orange tint observed. This organism is probably a variety of *A. Vinelandii* and was isolated from Soil Culture No. 16. This form was universally present in those original soil cultures described as showing a "beautiful, white, glistening membrane," and is apparently quite widely distributed in these island soils (see description of Soil Culture No. 6, above). The other unidentified member of this group is almost identical in appearance and in cultural characteristics with "B. 29 n. sp." derived from an Egyptian soil, a micro-photograph of which is to be seen in "Centralblatt für Bakteriologie," (etc.), Bd. 44, 1915, opposite page 511. A brief description of this species as found in the soil from the Hawaiian Commercial and Sugar Co., Puunene, Maui (Soil Culture No. 29) is as follows:

Shape: Oval to bacillus shaped; rounded ends. Occur singly or in short chains.
Size: 1.4 μ wide by 2.6 to 2.8 μ long.
Staining Properties: Stains solidly with methylene blue or Bismark brown although the cell walls at the edge are usually a little darker. There are no apparent internal markings or granules as is the case with ***A. Chroococcum.***
Motility: None.
Spores: None found even in old slant cultures.
Mannite agar slant: Narrow filiform; edges wavy and beaded; surface flat; color white, old cultures slightly brownish tint; moist growth, slightly raised at edges; slow grower.
Colonies on mannite agar: White to grayish; raised; round with slightly beaded edge; moist and glistening; no internal markings.
Potata Slant: Very slow thin growth.
Mannite agar stab culture: Very little growth in depths; spreading growth at surface.
Nitrogen-fixing power: In soils, 4.0 mg. per gm. of mannite; in solution, 6.0 mg. per gm. of mannite.

At the end of 3 weeks' incubation period, all of the mixed cultures were analyzed for total nitrogen by Hibbard's modified Gunning method (2), with the results as set forth in Table II.

TABLE II

NITROGEN FIXATION IN MANNITE SOLUTIONS BY 30 HAWAIIAN SOILS

Lab. No.	c.c. N/10 HCl used	N. found mg.	N. in 1 gm. Soil. mg.	N. fixed per culture. mg.	N. fixed per gm. mannite. mg.
1	11.4	16.0	8.7	7.3	3.65
2	17.9	25.1	10.0	15.1	7.55
3	9.1	12.7	5.2	7.5	3.75
4	8.4	11.8	5.4	6.4	3.20
5	13.5	18.9	3.5	15.4	7.70
6	13.8	19.3	3.9	15.4	7.70
7	8.4	11.8	4.1	7.7	3.85
8	13.7	19.2	6.2	13.0	6.50
9	9.8	13.7	6.0	7.7	3.85
10	10.4	14.6	4.0	10.6	5.30
11	9.9	13.9	4.3	9.6	4.80
12	11.3	15.8	7.4	8.4	4.20
13	12.8	17.9	7.4	10.5	5.25
14	9.3	13.0	5.3	7.7	3.85
15	10.6	14.8	5.7	9.1	4.55
16	22.5	31.5	4.2	27.3	13.65
17	15.6	21.8	1.8	20.0	10.00
18	14.8	20.7	3.3	17.4	8.70
19	12.4	17.4	1.3	16.1	8.05
20	9.3	13.0	0.7	12.3	6.15
21	11.8	16.5	1.5	15.0	7.50
22	10.4	14.6	1.7	12.9	6.45
23	11.9	16.7	2.2	14.5	7.25
24	9.5	13.3	1.0	12.3	6.15
25	4.2	5.9	1.0	4.9	2.45
26	6.0	8.4	1.9	6.5	3.25
27	6.0	8.4	2.1	6.3	3.15
28	10.7	15.0	3.1	11.9	5.95
29	7.4	10.4	2.7	7.7	3.85
30	7.8	10.9	1.7	9.2	4.60

A few interesting facts are brought out in a study of this table. As was pointed out by Lipman and Burgess (1), there seems to be a relation, at least in many cases, between the percentage of nitrogen in a given soil and that soil's powers of non-symbiotic nitrogen fixation; the higher the total nitrogen content, the lower the fixation, and vice versa. As is well known, the soils on the island of Hawaii run far higher in total nitrogen than do the soils from any of the other islands, while, generally speaking, those of Oahu, except on the windward side, are among the lowest. A glance at Table II shows that, taken by and large, the first fifteen soils (from Hawaii) show a lower fixation as well as a much higher total nitrogen content than do the seven soils which come from Oahu. This, however, does not hold true for the samples from the islands of Maui and Kauai, where fixing powers are the lowest of all. The exception here apparently proves the rule, for Soil No. 16 from the Kukaiau Plantation, carrying over 0.4 per cent total nitrogen, gives us the highest fixation of all, i. e. 13.65 mg. of nitrogen per gram of mannite consumed.

Of the thirty soil samples included in this work but five appeared to be absolutely devoid of Azotobacter growth. These were Nos. 4, 7, 9, 14 and 27. In these cultures, nevertheless, some fixation is recorded which indicates that probably certain Clostridium forms are also fairly prevalent here. It is further worthy of mention that the fixation of nitrogen in these five cultures, although from widely separated districts, is practically identical (varying from 3.15 to 3.85 mg. only), indicating a common organism.

While the amounts of nitrogen fixed in these mixed solution cultures are in no case exceptionally high, they are, taken by and large, far larger than the writer had anticipated from his earlier work reported elsewhere.

The four species of Azotobacter above isolated and described were now examined regarding their abilities to fix nitrogen in pure cultures, both in solutions and in soils. Young mannite agar slants (3 to 4 days old) were used in making the bacterial suspensions. This was done by pouring 10 c.c. of sterile tap water over the growth on an agar slant and working it up with a sterile platinum needle. All of the suspensions were fairly cloudy in appearance. Amounts of 1 c.c. each of these were used to inoculate 100-c.c. portions of the above sterile mannite solution in 500-c.c. flasks. Other 1-c.c. portions were used to inoculate 100-gm. portions of the station soil in covered tumblers which had previously been sterilized for about 3 hours at 15 pounds, and had each received 2 gm. of mannite after being cooled. The solution cultures only were run in duplicate. After incubating 3 weeks at 28° C., total nitrogen determinations were made by the method used above with the results as set forth in Table III.

A study of this table reveals the interesting fact that, contrary to custom, more nitrogen was fixed by these organisms in solutions than in soils, with but the one exception, *A. Vinelandii*, n. var.? In this culture,

however, a fine white mold growth was noticed toward the latter part of the incubation period which, to a certain extent, vitiates the result. Whether or not the soil used suffered so serious a change during sterilization as to be rendered unfit for Azotobacter growth, or whether these microörganisms work to better advantage in solutions than in soils, are questions which cannot now be answered. The pure cultures in solution all gave beautiful membranes. The first was light brown in color; the second, orange; the third, white; and the last, a very thin grayish film.

It was intended at the inception of this work to run all of the soils for nitrogen fixation by the "beaker method," in which the original soil plus mannite is used as its own culture medium, but, due to the press of other work, this has not been found possible. It was further intended to have micro-photographs taken of any unusual Azotobacter forms, should any be found, but, the station photographer being absent on a six months' vacation has caused us to abandon this also.

TABLE III

NITROGEN FIXATION BY PURE CULTURES OF AZOTOBACTER OBTAINED FROM HAWAIIAN SOILS; IN SOLUTIONS AND IN SOILS

SOLUTION CULTURES

Organism	c.c. N/10 HCl used	N. found per culture. mg.	N. fixed per gm. mannite. mg.	Average mg.
A. chroococcum, var...	6.8	9.5	4.75	4.62
A. chroococcum, var...	6.4	9.0	4.50	
A. Vinelandii, n. var?.	8.0	11.2	5.60	5.80
A. Vinelandii, n. var?.	8.6	12.0	6.00	
A. Vinelandii, var.....	12.2	17.1	8.55	8.57
A. Vinelandii, var.....	12.3	17.2	8.60	
"B. 29 ?".....	8.6	12.0	6.00	6.00
"B 29 ?".....	Lost	....	....	....

SOIL CULTURES

Organism	c.c. N/10 HCl used per 20 gm. soil	N. found mg.	N. found, blank subtracted. mg.	N. fixed per gm. mannite. mg.
A. chroococcum, var...	27.2	38.1	2.0	5.00
A. Vinelandii, var.....	27.2	38.1	2.0	5.00
A. Vinelandii, n. var?.	[1]28.4	39.8	3.7	8.75
"B. 29 ?".....	26.9	37.7	1.6	4.00
Uninoculated soil	25.8	36.1	...	....

[1] Some mold growth was noticed in this culture.

Remembering that the Hawaiian Islands are over 2000 miles from the nearest land area of any extent, it might, at first thought, seem hard to account for the prevalence of Azotobacter as noted in the work above reported. There are several ways, however, whereby to account for their possible introduction and distribution here. Hawaii is a land of heavy fertilization. The forty-odd sugar plantations annually expend about

three million dollars for mixed and single element commercial fertilizers, considerable of which comes from the Pacific coast (San Francisco chiefly). Fine soil is sometimes used as a "filler" in these "mixed goods" and, as this is never sterilized, it is conceivable that it might carry Azotobacter species. Dried blood, guano and tankage are also shipped here in considerable quantity, which may also be contaminated with these microörganisms. During recent years, green manuring with legumes has been quite extensively practiced on several of the larger plantations. These legume seeds have been imported from various parts of the world and might occasionally harbor Azotobacter. Be that as it may, we are sure of one fact: Azotobacter are quite universally distributed in Hawaiian soils.

Summary

1. Thirty soils from different localities on the four large islands of the Hawaiian Group were examined as to their Azotobacter content. Their abilities to fix nitrogen in mannite solutions are given.

2. Only five soils were noted which failed to show Azotobacter growth in solutions.

3. Four different forms of Azotobacter were isolated in pure cultures, described, and their abilities to fix nitrogen in solutions and in soils given.

4. A possible explanation of the introduction and distribution of Azotobacter in these isolated island soils is advanced.

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THE EFFECT OF TIME AND DEPTH OF CULTIVATING A WHEAT SEED-BED UPON BACTERIAL ACTIVITY IN THE SOIL[1]

By

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Before the American Society of Agronomy 1914, L. E. Call (1) of the Kansas Agricultural Experiment Station, presented a very interesting paper concerning the effects that different methods of preparing a seed-bed for wheat have upon yield, soil moisture, and nitrates.

The principal fact with which we are concerned, brought out in this paper, is the very marked effects that the various experimental methods have upon the accumulation of nitrates. So striking and consistent have the results been for the past several years that a careful study of the problem was deemed advisable. A study of the bacteriological factors involved was given the writer.

The project, as conducted by the Department of Agronomy, consists in comparing eleven different methods of preparing seed beds. A detailed description of the various methods, with results secured, etc., is given in the above mentioned paper. The major points of comparison have been time of preparation and the depth of culture. The dates of preparation vary from July 15, to seeding time, about October 1. The depth of culture varies from discing to plowing 7 inches deep. In general, the effect upon nitrate accumulation has been that early and deep preparations give materially higher nitrate contents at seeding time than do late and shallow. A few specific examples from Call's paper will suffice to show the influence of these two factors.

Plot No. 1	disced	Oct. 1,	22.43 lbs.	NO_3 per acre.
Plot No. 14	plowed 3 in. deep	Sept. 15,	57.30 lbs.	NO_3 per acre
Plot No. 15	plowed 3 in. deep	July 15,	517.01 lbs.	NO_3 per acre.
Plot No. 13	plowed 7 in. deep	Sept. 15,	76.83 lbs.	NO_3 per acre.
Plot No. 10	plowed 7 in deep	Aug. 15,	255.76 lbs.	NO_3 per acre.
Plot No. 9	plowed 7 in. deep	July 15,	407.94 lbs.	NO_3 per acre.

[1] Contribution from the Research Laboratory in Soil Biology, Kansas Agricultural Experiment Station.

An abstract of this paper was read before the Society of American Bacteriologists, 1915 meeting. Received for publication May 22, 1916.

From these data it is evident that some very important factors must be influencing the nitrate accumulation in the various plots.

For the bacteriological investigations it was deemed advisable to restrict the studies to a very limited number of plots. If the factors operating in the plots studied could be ascertained an application of similar methods to the whole series would be undertaken, provided such seemed advisable.

As representative of the extremes, both in methods of preparation and in results secured, so far as yield and nitrates are concerned, we chose as plots upon which to work Nos. 1 and 9. The treatment of No. 1 has been thorough discing at seeding time (about Oct. 1). The treatment of No. 9 has been plowing 7 inches deep July 15, and cultivating as often thereafter as necessary to insure a good seed-bed. For the four seasons '11, '12, '13 and '15, No. 1 has produced an average of 6.9 bushels of wheat per acre, while No. 9 has produced 27.59 bushels. The nitrate content for the four preceding seasons at the last analysis was for No. 1, 42.95 pounds of NO_3 per acre, and for No. 9, 318.69 pounds. From these data it is evident either that there has been much more NO_3 formed in No. 9, or that much has disappeared from No. 1. The only evident source of loss existing in No. 1 and not present in No. 9, is in the growth of weeds taking place prior to cultivation. Analysis has shown that in extreme cases this can account for only a small portion of the observed differences. It would seem then that those plots showing high NO_3 content actually form larger quantities. We have, therefore, for two seasons been directing our attention toward the nitrate forming power of soil from the two plots under study.

Experimental Data

The experiments given below include only a very few of those that have been carried out. An effort has been made to select representative experiments. At a later date a more complete report will be published.

Ammonia Forming Power

The relative ability of soil, from the two plots under study, to liberate ammonia from organic nitrogenous compounds is shown in Table I. Soil for the experiments here given was collected by means of a 2-inch soil auger. Twelve cores to a depth of 12 inches were drawn from each plot. Care was used to prevent, as far as possible, outside contamination.

The soil was brought to the laboratory, passed through a 3-mm. sieve, and thoroughly mixed. The moisture content and water holding capacity were determined. Four samples, the equivalent of 100 gm. dry soil, were then weighed. To two each of these cottonseed meal and dried-blood containing 60 mg. nitrogen were added, thoroughly mixed, and the whole placed in a 500-c.c. wide mouth bottle. The water content was made up

to optimum (2/3 saturation), the bottle plugged loosely with cotton, and incubated at room temperature 7 days. The ammonia was determined by direct distillation in presence of magnesium oxide. If the ammonia recovered by such means is any indication of the relative ammonifying power of the respective floras, it is evident that the difference in nitrate accumulation cannot be ascribed to a difference in this phenomenon. Further, repeated measurements of the ammonia content of the soils as they came from the field, revealed no appreciable difference. This was true whether such determinations were made by direct distillation or by the aeration method of Potter and Snyder (4).

TABLE I

THE PRODUCTION OF AMMONIA IN SOIL FROM PLOTS NO. 1 AND NO. 9: MG. NITROGEN RECOVERED AS AMMONIA PER 100 GM. OF SOIL AFTER ONE WEEK INCUBATION

Date	60 mg. Nitrogen as C. S. M.				60 mg. Nitrogen as D. B.	
	Soil Plot No. 1		Soil Plot No. 9		Soil Plot No. 1	Soil Plot No. 9
Oct. 23, '14.....	26.43 mg.		29.01 mg.		15.30 mg.	14.76 mg.
Nov. 7, '14.....	28.07 mg.		30.69 mg.		7.14 mg.	6.36 mg.
	A	B	A	B		
Nov. 21, '14.....	26.15 mg.	29.82 mg	26.82 mg.	30.06 mg.		

C. S. M.—Cottonseed Meal. D. B.—Dried Blood.
A.—Surface three inches. B.—5th to 7th inches inclusive.

Nitrate Forming Power

Experiments similar to those described above have been conducted to ascertain the nitrate forming power. Table II contains a description of treatment, etc., with the averages of three such determinations. The soil for these experiments was collected October 7, October 23, and November 7, 1914. Samples were incubated for four weeks, the water lost by evaporation being replaced from time to time. Nitrates were then determined by the phenol-di-sulphonic acid method as modified by Lipman and Sharp (2).

The results here presented indicate that in the absence of any addition of nitrogen, either with or without an addition of calcium carbonate, Plot No. 1 exhibits a materially higher nitrate forming power. On the other hand plot No. 9 exhibits a materially higher nitrate forming power when nitrogen as ammonium sulphate was added with calcium carbonate, and when nitrogen in form of cottonseed meal and dried blood was added in absence of calcium carbonate. In all other instances the differences are insignificant. A careful study of the data from which this table was constructed reveals, however, consistent differences in only the first three instances mentioned, namely, when no nitrogen was added either in the

presence or absence of calcium carbonate, and when ammonium sulphate was added in the presence of calcium carbonate. In all other comparisons sometimes Plot No. 1 and sometimes Plot No. 9 gave higher results.

The data contained in Table II indicate very strongly that, if the nitrate nitrogen produced under the experimental conditions here used gives any measure of the relative nitrate producing power, Plot No. 1 certainly contains as active a flora as No. 9. It is only just to add that in a number of experiments carried out in a similar manner, except that the nitrate content was measured at varying intervals, Plot No. 9 gave usually a somewhat more rapid accumulation during the early stages of incubation. However, such large quantities were in all cases formed in soil from Plot No. 1, as to show conclusively that the low accumulation under field conditions could not be attributed to a potentially weak flora. In addition there is absolutely no evidence to indicate a less active flora in the soil of Plot No. 1 when no nitrogen was added. Further, as pointed out above we have never been able to detect, either under field or laboratory conditions, a greater accumulation of ammonia in the soil of Plot No. 1 than in that of Plot No. 9.

TABLE II

THE PRODUCTION OF NITRATES IN SOIL FROM PLOTS NO. 1 AND NO. 9, INCUBATION FOUR WEEKS: AVERAGES OF DETERMINATIONS MADE ON DUPLICATE SAMPLES OCT. 7, OCT. 23, AND NOV. 7, 1914

Treatment		Mg. NO_3 per 100 gm. Soil	
60 mg. Nitrogen as	$CaCO_3$ gm.	Soil Plot No. 1 mg.	Soil Plot No. 9 mg.
0	0	2.93	3.26
0	1	8.43	6.85
Cottonseed Meal	0	67.02	76.59
Cottonseed Meal	1	153.89	154.45
Dried Blood	0	29.96	36.36
Dried Blood	1	102.33	103.31
Ammonium Sulphate	0	21.11	21.83
Ammonium Sulphate	1	155.23	190.51

The nitrate forming power in solution, as so vigorously recommended by Löhnis and Green (3), has also been tested. Data secured according to this method, for Sept. 14, 1915, are given below. The figures represent milligrams nitrogen converted into NO_3.

Incubation Period	10 days	28 days
Inoculum Soil of Plot No. 1	0.95 mg.	8.41 mg.
Inoculum Soil of Plot No. 9	1.36 mg.	8.47 mg.

Soil from Plot No. 1 has also been inoculated with soil from Plot No. 9 to see if the introduction of organisms from No. 9 would have any influence on the accumulation of nitrates. The results secured Sept. 14, 1915 are given in Table III. In both these last two mentioned experiments additional evidence is furnished to show that there is but slight

difference in the nitrifying floras of the two plots under study. Similar studies, giving similar results, have been made on other plots in the series.

TABLE III

THE EFFECT OF INOCULATING SOIL FROM PLOT NO. 1 WITH SOIL FROM PLOT NO. 9: MG. NO_3 RECOVERED PER 100 GM. SOIL

Soil	Inoculum	60 mg. Nitrogen as	$CaCO_3$ gm.	After 4 weeks mg.	After 10 weeks mg.
1	0	0	0	3.32	8.20
1	1	0	0	4.00	7.50
1	9	0	0	4.00	8.20
1	0	$(NH_4)_2SO_4$	1	.....	225.00
1	1	$(NH_4)_2SO_4$	1	133.30	200.00
1	9	$(NH_4)_2SO_4$	1	150.00	200.00

While no differences in the activity of the organisms could be detected in the above experiments, it was thought that possibly by sampling the soil at different depths and making similar studies, differences might be noticed. Accordingly, samples have been taken at varying depths down to 12 inches. Under our conditions very small quantities of nitrates are formed at lower depths. The results of one such analysis are given in Table IV. Soil for these experiments was collected August 20, 1915. A

TABLE IV

SHOWING NITRATE FORMATION IN SOIL FROM DIFFERENT DEPTHS OF PLOTS NO. 1 AND NO 9: Mg. NO_3 RECOVERED PER 100 GM. SOIL

Weeks Incubated	Treatment		Soil Plot No. 1				Soil Plot No. 9			
	60 mg. Nitrogen as	$CaCO_3$ gm.	A	B	C	D	A	B	C	D
0	0	1	1.42	1.24	1.24	1.24	2.80	2.34	2.40	1.60
2	0	1	5.40	3.00	2.25	1.50	9.00	9.60	7.80	2.49
4	0	1	18.00	2.64	2.49	1.29	13.80	8.59	9.60	4.20
8	0	1	30.00	13.86	5.28	3.00	11.40	13.80	15.00	6.42
16	0	1	40.00	19.40	15.00	9.00	16.35	20.00	20.00	7.70
1	Cottonseed Meal	1	4.00	2.00	1.60	1.00	2.73	2.73	2.10	0.84
2	Cottonseed Meal	1	19.50	8.16	4.17	1.50	16.00	18.50	13.20	3.60
4	Cottonseed Meal	1	128.40	114.00	120.00	114.00	116.40	112.80	118.00	118.00
8	Cottonseed Meal	1	138.00	120.00	120.00	128.40	120.00	123.60	128.40	123.40
1	Ammonium Sulphate	1	6.40	2.66	2.00	1.42	7.20	6.90	5.20	2.00
2	Ammonium Sulphate	1	15.30	7.20	4.50	2.82	15.80	18.70	14.00	5.60
4	Ammonium Sulphate	1	189.60	114.00	133.30	60.00	180.00	180.00	180.00	75.00
8	Ammonium Sulphate	1	200.00	180.00	180.00	180.00	180.00	200.00	200.00	180.00

cylinder 5 inches in diameter was driven into the soil 12 inches deep, then was dug out and the core of soil divided into four equal parts. Five such cores were taken from each plot and the corresponding sections thoroughly mixed. Otherwise, the experiments were carried out as described earlier in the paper.

Several very interesting facts are to be noted in the data contained in Table IV. One of the most striking is the very rapid accumulation of nitrates in the surface soil of Plot No. 1, when no nitrogen was added. Below the surface section the accumulation fell off rather rapidly. On the other hand, the second and third layers of Plot No. 9 show just as rapid accumulation as the surface layer. Not until the fourth layer is reached do we note a decrease in nitrate accumulation. The results of this particular experiment are not nearly so marked in this respect as many others that have been carried out. It is reported only because it covers a wider range than the other experiments covering the same field. The results of a similar experiment, which was continued for a year, are graphically shown in figure 1. Soil for this experiment was collected Nov. 21, 1914. Here the second layers of soil differ from those in the experiment reported above in that they include the soil from the fifth to the seventh inches inclusive, instead of that from the fourth to the sixth. We shall take up a discussion of this particular phenomenon later.

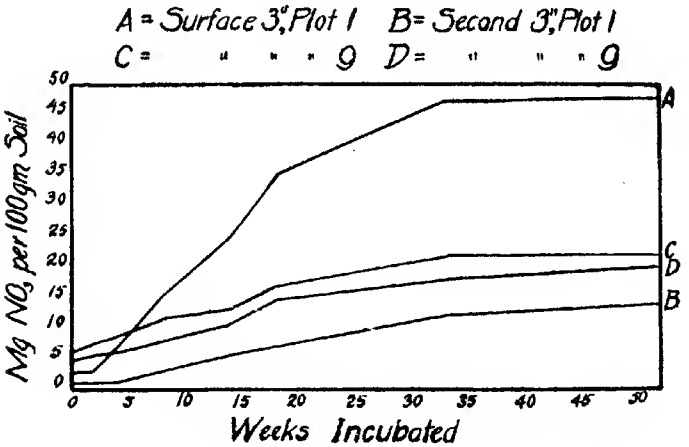

Fig. 1.—Diagram showing NO_3 accumulation in soil from different depths.

Returning to Table IV, we note that where nitrogen was added, somewhat similar results were obtained after one and after two weeks' incubation. That is, the NO_3 accumulation fell off rather rapidly immediately below the stirred area. Such differences, however, were in almost all instances eliminated by a longer incubation period. The question naturally arises as to whether this difference is due to some physical, chemical, or biological difference brought about by cultivation. We are at present in-

terested only in biological factors. If the differences are due to variations in the nitrifying floras, there are two ways in which we might detect the same. Either by introducing the respective floras into a standard solution and measuring their activity, or by cross inoculating experiments. Experiments have been carried out by both methods and yielded alike negative results, so far as exhibiting differences between the two plots is concerned.

In solution the following quantities of nitrogen, expressed as milligrams converted into NO_3 during four weeks' incubation, were found:

	Plot No. 1	Plot No. 9
1st 3 inches	3.69 mg.	4.42 mg.
2nd 3 inches	3.07 mg.	3.30 mg.
3rd 3 inches	2.31 mg.	2.29 mg.
4th 3 inches	4.24 mg.	4.11 mg.
Average	3.33 mg.	3.53 mg.

These results, together with others secured under similar conditions, give no indication either of a material difference in the nitrifying floras of the different plots, or of the different layers of an individual plot.

An extensive series of cross inoculation experiments was not conducted at this particular date. The results reported below were secured from soil collected May 3, 1915. Duplicate samples from each layer of both plots were inoculated from every other layer of both plots, giving a total of 72 samples. Half the samples were incubated 4 weeks, the other half, 12 weeks. Only the average nitrates, expressed in milligrams NO_3 recovered from all samples inoculated from the same source, are given.

	Plot No. 1			Plot No. 9		
Inoculum	A	B	C	A	B	C
Mg. NO_3	10.3	10.3	9.9	10.4	10.4	10.4

(A—1 to 3 inches inclusive; B—5 to 7 inches inclusive; C—8 to 12 inches inclusive). When only one experiment is considered, the differences here shown are well within the experimental error.

We have, therefore, been unable to detect any consistent differences in the nitrifying power of the respective floras which could, in our opinion, explain the large differences observed in nitrate accumulation under field conditions. This being true, the controlling factors must be other than biological. We have undertaken some experimental work studying the physical and chemical factors that might be influencing the activity of the nitrifying organisms. Work along these lines is by no means complete; still, certain facts brought out may throw some light on the general problem.

From the evidence in hand, and in part presented above, two points of importance seem to be clearly demonstrated. First, transferring the soil from field to experimental conditions eliminates the differences normally

existant. Second, the surface soil of Plot No. 1 possesses the ability to transform into nitrate nitrogen much more of its native nitrogen than does any other layer of either plot, or at least at a much more rapid rate. As accurately as we, by our present methods, are able to detect, these facts cannot be explained solely upon a difference in the respective floras either under their natural or under laboratory conditions.

The most evident changes brought about in transferring the soil from field to experimental conditions are: stirring, aerating, changing the temperature, and changing the moisture content. It is a common observation that stirring soils, apparently aside from all other influences, materially accelerates bacterial activity, and no doubt, has an influence on the phenomon under consideration. If this were the major factor, however, those layers of soil normally remaining unstirred i.e.—4 to 12 inches inclusive of No. 1, and 8 to 12 inches inclusive of No. 9, should show a greater response under the new environment. The evidence (see Table IV) does not bear this out. What has been said with regard to stirring also applies to aerating. It is hardly conceivable, though, that the surface soil of Plot No. 1 lacks aeration, yet this is the soil which gives the greatest response under laboratory conditions. In fact, Table IV shows that in all cases those soils receiving better aeration under field conditions give higher nitrate formation under laboratory conditions.

We believe temperature changes are a negative factor, since under field conditions the differences could be only very slight and in laboratory all were subjected to the same temperature.

As stated above, the soils in all experiments herein reported were made up to optimum moisture conditions, i.e. two-thirds saturated. In this respect they were materially changed from field conditions. Figure 2 illustrates the effect upon nitrate accumulation produced by varying the moisture content of soil from the experimental field. From this it can be seen that an increase of 1 per cent moisture, at or near the minimum for nitrification, may cause an increase of 100 per cent in nitrate production. If moisture is an influencing factor, however, it necessarily implies a variation of moisture content under field conditions. We have collected but little data on the moisture content of the soils under field conditions, but, through the courtesy of the Department of Agronomy, the data collected by them during the past seven years have been made available for study. They have sampled the various plots monthly in foot sections and made moisture determinations. Since the variations in nitrate content are largely brought about during August and September and almost entirely in the first foot of soil, we have confined our studies to the data collected during those two months, and for the first foot sections. It could not be expected that such meagre data would give us a clear insight into the relation existing between moisture content and nitrate pro-

duction. However, a remarkably close agreement can be traced for some seasons. Figure 3 illustrates graphically the correlation between the two factors for the season 1911. Similar relations, though not so close, can be traced for all other years for which data are available, except 1913.

It will be remembered that 1913 was omitted from the averages of yields and nitrates present in plots No. 1 and No. 9 given earlier in the paper. This was done because of the uniform results secured for that season. The yield was on Plot No. 1, 21.83 bushels, and on No. 9, 25.83 bushels of wheat per acre, a difference of only 4 bushels, while checks showed showed a variation of 6.25 bushels. The difference in nitrate content was at seeding time only 3 pounds per acre in favor of No. 9. The greatest variation in NO_3 content in the whole series was between

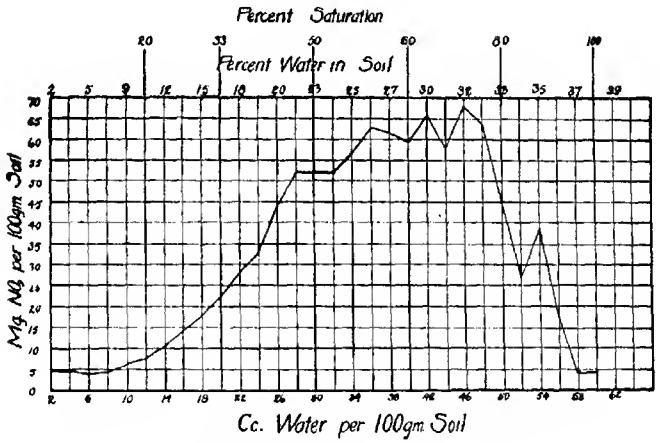

Fig. 2.—Diagram showing the effect of varying water content upon NO_3 formation in soil from experiment field.

checks. The same is true regarding moisture content. In other words, the whole series gave negative results for this season. A glance at figure 4, illustrating graphically moisture and nitrate content for 1913, shows absolutely no correlation between moisture content during August and September and nitrate content. In figures 3 and 4 the moisture lines give the average per cent of moisture actually available for nitrifying organisms when the August and September analyses were made. The nitrate lines represent pounds of NO_3 per acre three feet at the October analysis. We do not wish to be understood as saying that the large differences observed under field conditions can be entireely explained upon a moisture content basis. We do believe, however, that the few facts presented above are of considerable significance.

Turning our attention for a moment to the differences exhibited under laboratory conditions between the various layers of soil as illustrated in figure 1, it may be asked, how can these be explained? No evidence indicating a lack of active organisms in the lower layer soil of Plot No. 1 has been secured. The differences then must be due either to an inhibiting agent in the lower layer or to a difference, either in quantity or quality, of nitrogen present in the two layers. The fact that added nitrogen in any readily available form has been rapidly nitrified in the lower layer would seem to preclude the first possibility. As to the second possibility, it was found that the surface layer contained approximately 100 parts per million, or actually 100 pounds per acre, more nitrogen than the sec-

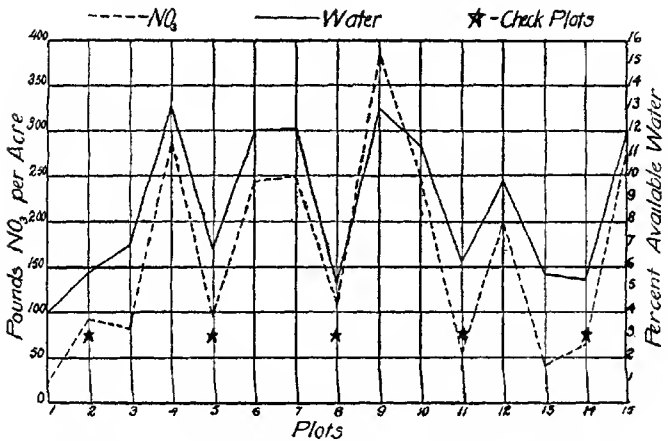

Fig. 3.—Diagram showing the relation between available water and NO_3 content under field conditions, 1911.

ond. There are, however, approximately 1500 parts per million nitrogen in the second layer, and unless this 100 parts per million exists in a different form or condition it would appear relatively insignificant. Besides, the second layer soil of Plot No. 1 contained slightly more nitrogen than either layer of Plot No. 9, yet, it failed to produce as much nitrates. As pointed out earlier, no difference could be detected in the ammonia content. This is rather significant since ammonia is the immediate forerunner of nitrates. Observations indicated a much larger quantity of undecomposed organic matter in surface soil from Plot No. 1 than in any of the other layers under study. By an arbitrary specific gravity method of separating this organic matter it was found in the following quantities in the four soils:

Surface layer No. 1 13,500 parts per million
Surface layer No. 9 3,400 parts per million
Second layer No. 1 2,200 parts per million
Second layer No. 9 3,700 parts per million

The nitrogen contents of this organic matter were in practically the same proportions. The excess of undecomposed organic matter in the surface layer of Plot No. 1, therefore, contained approximately 100 pounds of nitrogen, a figure just equal to the difference in total nitrogen between the two layers. A glance at figure 2 will show that when the NO_3 content of the surface soil of Plot No. 1 exceeds the second layer by approximately 100 pounds of nitrogen, the production in the two layers runs almost parallel (32nd to 52nd week). In other words, it appears

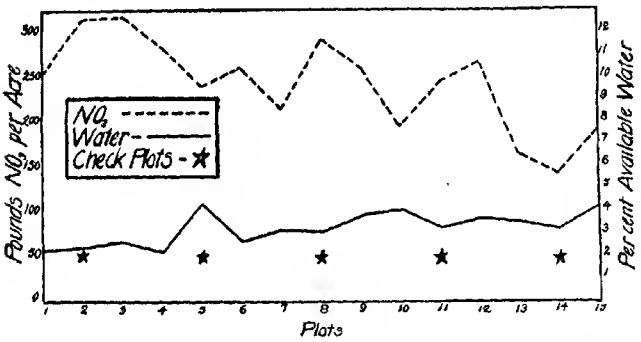

Fig. 4.—Diagram showing the relation between available water and NO_3 content under field conditions, 1913.

that something has prevented the decomposition of organic matter in the surface soil of Plot No. 1 under field conditions. This is, no doubt, due to the fact that the cultural method followed on this plot, i. e. discing, leaves the organic matter on or near the surface where, because of the normally dry conditions during the summer and fall months, decomposition is impossible. On the other hand, deep plowing incorporates the organic matter throughout the first 7 inches. More moisture being present, both because of depth and early culture, decomposition is made possible.

Summary

As a summary we may state that the evidence here submitted indicates that:

1. The differences in nitrate content reported by Call cannot be attributed to a difference in the bacterial content.

2. Some non-biological condition existing in certain plots, under field conditions, prevents the normal activity of the bacterial flora.

3. Among the factors controlling bacterial activity the available moisture probably plays a paramount rôle.

ACKNOWLEDGEMENT

The writer wishes to express his appreciation of the valuable help rendered by the Department of Agronomy, through Prof. L. E. Call, in furnishing such data as have been used relative to yields, and moisture and nitrate content under field conditions.

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